

ANNUAL REPORT

**Division of Intramural Research Programs
National Institute of Mental Health**

October 1, 1987 - September 30, 1988

**VOLUME II PART 2
INDIVIDUAL PROJECT REPORTS**

**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Alcohol, Drug Abuse, and Mental Health Administration
National Institute of Mental Health
Division of Intramural Research Programs**

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DIVISION OF INTRAMURAL RESEARCH PROGRAMS

NATIONAL INSTITUTE OF MENTAL HEALTH

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INDIVIDUAL PROJECT REPORTS**

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Neural mechanisms of cognitive memory and habit formation

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

EVERY FORM OF WORK (Use standard unabbreviated type. Do not exceed line space provided.)

Every sensory modality in the macaque is served by a series of cortical stations, each of which processes the sensory signal in turn. Signals in the later stations, located in the anterior temporo-insular cortex, can activate a circuit that runs through the limbic system to the modulatory neurochemical systems (e.g. cholinergic, noradrenergic, etc.) and back to the sensory cortical stations. We have proposed that as a result of the action of this circuit on neurochemical release in sensory cortex, some of the neurons whose signals have just represented the sensory stimulus become linked together in a cell assembly that serves as the stored representation of that stimulus. Recognition, say of an object, occurs when an assembly formed on a first presentation of the object is reactivated by its re-presentation on a second occasion. Also, once formed, that assembly can be linked to assemblies representing other stimuli and other events, such as a food reward or a location, thereby investing the recognized object with meaning. The linkage involved in object-reward association appears to be mediated mainly by a limbo-neurochemical circuit running through the amygdala, the medial dorsal thalamic nucleus, orbital frontal cortex, and the basal nucleus of Meynert. Similarly, the linkage involved in object-place association seems to be mediated mainly by a second, parallel limbo-neurochemical circuit running through the hippocampus, the anterior thalamic nuclei, cingulate cortex, and the medial septal and diagonal band nuclei. Each of these circuits has reciprocal connections with one pair or the other of the assemblies described above. Thus, if these circuits have been activated, the sight of the object on a second occasion can lead not only to its recognition but also to recall of both the food reward and the spatial location with which the object had been associated. Recognition and recall are two forms of cognitive memory, both of which can be distinguished from habit formation. The latter form of learning involves stimulus-response association specifically, and we have proposed that such learning depends largely on interactions between the cerebral cortex and the basal ganglia.

PROJECT DESCRIPTION:

The objective of the studies in this project is to delineate the neural system underlying memory formation in the monkey and to differentiate it from the neural system that underlies habit formation. The methods used include behavioral analyses of the effects of selective cerebral ablations and disconnections combined with anatomical analyses of functional neural pathways. The rationale and design of the studies are often based directly on information derived from other projects in this laboratory, many of which deal with the pathways for, and the mechanisms of, stimulus processing and encoding. The results from these and other projects suggest that the sensory system for each modality is composed of two hierarchically organized corticocortical pathways, one directed ventrally to the temporal lobe limbic system and concerned with object perception, and the other directed dorsally to the frontal lobe motor system and concerned with spatial perception. The ultimate goal of this project is to determine how object and spatial perceptions in the different modalities are formed into memories, how these different memories are associated with each other, how they evoke emotions and motor acts, and how they lead not only to these cognitive events but also to habit formation. Our progress in understanding each of these processes will be described in turn.

(1) Recognition memory

Previous work has indicated that visual recognition memory (assessed by delayed nonmatching-to-sample with trial-unique objects) is mediated by a cortico-limbo-thalamic system composed of two largely separate circuits arranged in parallel. One of these circuits consists of the amygdala, amygdalofugal pathways, and the magnocellular portion of the mediodorsal nucleus (MDmc), and the other consists of the hippocampus, fornix, and anterior nuclear complex of the thalamus (Ant N). The reason for believing that these two circuits operate in parallel is that damage to the amygdalar and hippocampal circuits at each stage in the system (i.e. medial temporal lobe, limbo-thalamic pathways, and medial thalamus) causes a severe loss in recognition memory, but only when the two circuits are damaged in combination. Damage to just one of the two circuits leads to only mild recognition deficits, suggesting that either circuit can compensate for the other as far as recognition is concerned. Recent results indicate that the orbital frontal and anterior cingulate cortical zones, which are related anatomically to the amygdalar and hippocampal circuits, respectively, must also be removed in combination to produce a severe recognition deficit. Removal of either the orbital frontal or anterior cingulate cortex alone, or of prefrontal tissue outside this zone, produces little impairment. Thus, the ventromedial prefrontal region appears to constitute yet another stage in the limbic memory system. In an attempt to determine if the different stages have redundant memory functions, we have begun an experiment that examines the effects on recognition memory of combined ablations of tissue at different stages within a circuit. Preliminary data suggest that combined hippocampal and cingulate cortical ablation leads to more severe memory deficits than ablation of the hippocampus alone. Likewise, combined ablation of the amygdala and orbital frontal cortex leads to a more severe deficit than ablation of the amygdala alone. These new results suggest that the prefrontal

cortical stage of each limbic circuit receives a secondary input from the other circuit.

Recent anatomical evidence has indicated that the bed nucleus of the stria terminalis (BNST) occupies an anatomical position within the amygdalar system that is comparable in some ways to that occupied by the mamillary bodies within the hippocampal system. That is, just as the hippocampal formation projects to the Ant N both directly and indirectly via the mamillary bodies, the amygdala projects to MDmc both directly and indirectly via the BNST. These particular relays between the medial temporal lobe and medial thalamus are not normally critical for recognition memory, since combined damage to the two relays yields only a mild impairment. They might have an important role, however, in the enhancement of memory by emotion, a possibility that we plan to explore in the near future.

Investigations of recognition memory in monkeys have largely been confined to vision. The one exception was an experiment from this laboratory showing that temporal lobe limbic structures are just as important for tactile as for visual recognition. Experiments in another project (MH 02037) have demonstrated that a cortical tactile processing pathway runs from the postcentral somatosensory cortex to the second somatosensory area, SII, from SII to the insular cortex, and, finally, from the insular cortex to the medial temporal region. These results suggest that the insular cortex could play a role in tactile recognition analogous to that played by the inferior temporal cortex (area TE) in visual recognition. To test this idea, we are comparing the effects of inferior temporal and insular lesions on both visual and tactile recognition. Our preliminary results indicate that monkeys with bilateral ablations of this cortex are severely impaired in tactile but not visual recognition of objects. Thus, the results suggest that the somatosensory system, like the visual system, interacts with the limbic system through a series of modality-specific areas. Besides pursuing the tactile recognition studies, we are continuing to explore behavioral methods for evaluating recognition memory in audition so that we can extend our behavioral investigations to this modality.

In addition to examining memory of stimulus quality in each sensory mode, we are interested in studying spatial memory. To this end, we have recently trained monkeys on spatial delayed nonmatching-to-sample in a T-maze, a task that evaluates recency memory for place. The results indicate that monkeys with fornix transections are severely impaired and monkeys with cingulate cortical ablations are mildly impaired relative to intact controls. In addition to indicating that the hippocampal system in monkeys is important for this kind of spatial memory, the experiment helps establish a firm link between primate and rodent memory studies, in that this particular spatial memory task, like most such tasks employed with rodents but unlike most employed with monkeys, involves locomotor responses.

(2) Anatomy of recognition memory

Although we have demonstrated the importance of cortical inputs to the limbic system in object recognition memory, we had not made as much progress in demonstrating the anatomical substrates responsible for the limbic feedback to the cortex that allows memories to be stored. A major hypothesis has been that the basal forebrain cholinergic system plays an important role in this process. But the relations of the basal forebrain to the medial temporal limbic areas and to the midline thalamus had not been completely described. Recent studies have addressed these issues.

Injections of tritiated amino acids were made into the hippocampal formation and amygdaloid complex in order to trace their inputs to the basal forebrain, with the following results. The hippocampal formation projects densely to the medial (Ch1), lateral, and dorsal septum, and to the Ch2 region. There were relatively sparse projections to restricted portions of Ch4 as well. Experiments in which the fornix was transected demonstrated that all of these projections ran through the fornix. The caudal hippocampus projects to the more medial septum, whereas the rostral hippocampus projects to the more lateral septum, although there is considerable overlap in the projections from these two regions. The regions of the basal forebrain and septum receiving hippocampal inputs project back to the hippocampus.

The projections from the amygdala do not overlap those from the hippocampus, but terminate instead in the Ch3 and Ch4 fields of the substantia innominata. In fact, the amygdala represents one of the major inputs to Ch4, which provides the major portion of the cholinergic input to the cerebral cortex. The amygdalar efferents arise from the medial, medial basal, magnocellular portion of the accessory basal, and the central nuclei. It is the more dorsal and medial portions of this group that receive the largest number of intrinsic amygdalar connections. The lateral nucleus, which is the site of termination of afferents from the major sensory systems, provides almost no outputs to the basal forebrain.

Because lesions of the medial thalamus lead to deficits in recognition memory like those caused by medial temporal damage, and because the medial and midline thalamic nuclei receive inputs from the hippocampal formation and amygdaloid complex, the structures occupying this region appear to be intimately involved in memory formation. To examine medial thalamic relations with the frontal cortex, we injected anterogradely transported tracers into medial thalamic nuclei and retrogradely transported tracers into various ventromedial prefrontal cortical areas. The results indicate that precallosal cortical areas 14, 25, and 32 all receive projections largely from dorsal MDmc. In the most anterior portions of areas 14 and 32 and in area 10, the projections arise from dorsal MDpc. Sub- and supra-callosal area 24 receives its major thalamic input from the anterior nuclear complex, mostly the anterior medial nucleus. Projections from the anterior nuclear complex extend to large portions of medial limbic cortex, including, in addition to area 24, areas 23, 25, and retrosplenial and entorhinal cortex.

In summary, the precallosal prefrontal cortical regions have access to amygdalar information via MDmc, while the sub- and supra-callosal prefrontal cortical regions have access to hippocampal information via the anterior nuclear complex. These projections could well form part of a cortico-limbo-thalamo-cortical feedback circuit involved in the formation of new memories.

(3) Associative memory

Our earlier work suggested that although the amygdalar and hippocampal systems contribute equally to recognition memory, they have selective roles in associative memory. The amygdala, but not the hippocampus, appears to be important for the association of object qualities from different sensory modalities. In contrast, the hippocampus, but not the amygdala, appears to be important for the association of object quality and place. To test these generalizations, monkeys are being trained on a visual-visual associative memory task. Preliminary results indicate that, compared to hippocampectomized monkeys, those with amygdalar ablations are retarded in relearning a preoperatively acquired set of visual-visual associations. Furthermore, monkeys with the combined amygdalo-hippocampal ablations are unable to relearn within the training limit of 5,000 trials. These same monkeys are able, however, to perform a delayed matching task employing the same stimulus set and the same short delays, so their impairment cannot be due to an inability to discriminate the stimuli or remember them for short periods of time. Interestingly, neither monkeys with amygdalar removals alone nor those with hippocampal removals alone appear to be impaired in learning new sets. The critical question now is whether monkeys with combined ablations of the amygdala and hippocampus will be impaired in learning new sets. If so, it would suggest that, in monkeys, the formation of arbitrary visual-visual associations, like paired-associate learning in humans, is a measure of cognitive memory to which both the amygdala and hippocampus contribute equally. Alternatively, a finding that monkeys with the combined ablation can learn new sets would suggest that some nonlimbic mechanism can contribute to intramodal stimulus-stimulus learning, a mechanism that might be related to the phenomenon of priming observed in amnesic humans.

Earlier, we had found that monkeys with hippocampal ablations, but not those with amygdalar ablations, were impaired on each of two trial types of a spatial memory task: (1) "object-place" trials, requiring one-trial memory for the association of an object and its location; and (2) "place" trials, a version of spatial delayed response requiring memory for location only. To determine whether the monkeys in that experiment failed the "place" trials because of interference from a strategy needed to remember the more difficult "object-place" trials, we trained naive monkeys on "place" trials only. Preoperatively, the monkeys scored 85 percent correct responses, whereas following bilateral hippocampectomy they achieved only 60 percent correct. The results indicate that a) impairment on the "place" trials in the earlier study was not due to interference from the "object-place" trials, and b) performance on this version of spatial delayed response, unlike performance on the classical version, depends critically on the hippocampus.

(4) Habit Formation

Whereas monkeys with limbic lesions generally exhibit poor memory on recognition and associative memory tasks they are able to learn certain types of object discriminations at a normal rate. For example, we have found that such monkeys can learn as rapidly as normal control animals to discriminate 20 pairs of objects presented concurrently, even with intertrial intervals lasting 24 hours. We have applied the label "habit formation" to this and other examples of preserved learning ability following limbic-system lesions. Normal monkeys trained on the discrimination test with 24-hour ITIs learn successive sets of object discrimination progressively faster. To investigate the basis of this phenomenon, we tested whether it is affected by limbic lesions. The results show that monkeys with combined amygdalo-hippocampal ablations show the same amount of improvement as normal monkeys, indicating that this type of learning set formation is supported by nonlimbic mechanisms.

In a separate LN project (MH 02039), we have been attempting to examine the role of the neostriatum in 24-hr-ITI learning by disrupting the entire nigrostriatal dopaminergic system with the selective neurotoxin MPTP. Because preliminary results with two different regimens of MPTP administration suggest that there is little effect, if any, at doses that do not also produce motor impairments, we are planning to test the effects of damaging selectively those portions of the neostriatum to which the cortical visual system projects (see LN project MH 02033).

Another behavioral paradigm that may provide a measure of the ability to acquire habits is delayed nonmatching-to-sample (DNMS). Although combined amygdalo-hippocampal removals in macaques severely impair their performance on DNMS when delays between sample and choice exceed about 10 seconds, they can master the task with shorter delays. Such mastery cannot depend on the formation of specific visual discrimination habits, because (a) a different pair of objects is used on every trial and (b) within a trial, the reinforcement contingencies for responses to the sample object are inconsistent. To master the task in the absence of the limbic system, the animal must be able to learn a rule, which requires, in turn, (i) suppression of specific stimulus-response habits, (ii) abstraction of sameness and difference from specific stimulus quality with the aid of immediate memory, and (iii) formation of a stimulus/difference-response habit. We have now found that if inferior prefrontal lesions (which produce a moderate DNMS impairment by themselves) are added to amygdalo-hippocampal lesions, monkeys lose the ability to perform DNMS even when the delays are less than 10 seconds. This finding suggests that the inferior prefrontal cortex serves one or more of the processes described above needed for rule learning, and that it does so by mediating a complex set of interactions between the inferior temporal cortex and the neostriatum, with both of which the inferior prefrontal cortex is directly connected. These data open up a new chapter in frontal lobe research, namely, the role of the prefrontal cortex in the formation of complex habits.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

In the process of investigating the role of various temporal lobe structures in the visual memory of monkeys, we obtained a result that is particularly exciting because it appears to solve the long-standing puzzle concerning the neuropathology underlying the syndrome of global anterograde amnesia in man. This syndrome, which is characterized by a profound inability to remember new experiences or acquire new information, has been attributed in the clinical literature to destruction of the hippocampus. Yet, attempts to duplicate this syndrome in monkeys by removal of the hippocampus alone have largely failed. What we have found in our studies is that if damage to the hippocampus is combined with damage to the amygdala then a profound memory loss does ensue. The discovery has not only resolved the discrepancy between clinical and experimental findings in nonhuman primates, but has also provided new insight into the neural substrates of memory. Specifically, it has led to the development of a hierarchical model of recognition and associative memory involving a cortico-limbo-neurochemical loop that may well serve as the foundation for all cognitive processes beyond perception, including thought. As we gain further understanding of the memory system, and how it differs from the noncognitive system for habit formation, we will inevitably gain a better understanding of thought and its breakdown in normal and abnormal behavior.

PROPOSED COURSE OF RESEARCH:

Having found severe object recognition losses in both vision and touch after lesions of the limbic system, we shall continue our attempts to devise tests of auditory recognition and visual spatial recognition, with the aim of determining whether the limbic system is indeed critical for recognition in all perceptual modalities. Also, further attempts will be made to differentiate between amygdalar and hippocampal contributions to associative memory, and we shall test whether any functional distinctions that apply to these temporal lobe structures are carried further through the thalamic, prefrontal, and neurochemical segments of the two limbic circuits. In addition, we shall continue our exploration of the neural basis of habit formation, with particular attention initially to the neostriatal and prefrontal targets of the occipitotemporal visual system.

PUBLICATIONS:

Aggleton, J.P., Friedman, D.P., and Mishkin, M. A comparison between the connections of the amygdala and hippocampus, with the basal forebrain in the macaque. Exp. Brain Res. 67: 556-568, 1987.

Bachevalier, J., and Mishkin, M. Mnemonic and neuropathological effects of occluding the posterior cerebral artery in Macaca mulatta. Neuropsychologia, in press.

Markowska, A.L., Olton, D.S., Murray, E.A. and Gaffan, D. A comparative analysis of the role of the fornix and cingulate cortex in memory: rats. Exp. Brain Res., in press.

Mishkin, M. and Phillips, R.R. A cortico-limbic memory path revealed through its disconnection. In C. Trevarthen (Ed.): Brain Circuits and Functions of the Mind: Festschrift for Roger Wilcott Sperry. Cambridge University Press, New York, in press.

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Nelson, R.B., Friedman, D.P., O'Neill, J.B., Mishkin, M., and Routtenberg, A. Gradients of protein kinase C substrate phosphorylation in primate visual system peak in visual memory storage areas. Brain Res. 416: 387-392, 1987.

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Phillips, R.R., Malamut, B.L. Bachevalier, J. and Mishkin, M. Dissociation of the effects of inferior temporal and limbic lesions on object discrimination learning with 24-h intertrial intervals. Behav. Brain Res. 27: 99-107, 1988.

Saunders, R.C. and Rosene, D.L. A comparison of the efferent connections of the amygdala and the hippocampus. I. Convergence in the entorhinal, prorhinal and perirhinal cortex. J. Comp. Neurol. 271: 153-184, 1988.

Saunders, R.C., Rosene, D.L., and Van Hoesen, G.W. A comparison of the efferent connections of the amygdala and the hippocampus. II. Reciprocal and non-reciprocal connections. J. Comp. Neurol. 271: 185-207, 1988.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02032-12 LN

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neural coding of visual stimuli in the awake monkey

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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Others:	L. Optican	Research Engineer	LSR NEI
	M. Mishkin	Chief	LN NIMH
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SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To study the mechanisms underlying visual perception we recorded the activity of ganglion cell fibers, the fibers that send visual information from the retina to the brain, and compared it with the recordings of single neurons in the lateral geniculate nucleus and primary visual cortex, the first two extraretinal stages of visual processing, and inferior temporal cortex, the last visual processing station in the cortex. The three sets of neuronal recordings all showed different temporal response patterns to different visual stimulus patterns. When neurons were analyzed as communication channels carrying information about visual stimuli in their responses, the response patterns seen could only be represented as the sum of several (3-6) simultaneous, independent patterns of activity. Three of these activity patterns were analyzed as a temporal code, and this code was found to contain twice as much information as that conveyed by the response strength, the usual measure of neuronal response. However, the ganglion cells fibers, which carry as much information as the neurons in subsequent stages, have substantially less information in the temporal modulation code than in the response strength code. Thus, temporal modulation carries a significantly greater proportion of visual information outside of the retina. Traditionally, it has been thought that information about multiple stimulus parameters, such as luminance, pattern, and duration of presentation, must be confounded in the neuronal responses. However, based on this multiplex-filter hypothesis, a new analysis of the neuronal responses of primary visual cortex led to the discovery that information about each of these parameters is carried separately in the response. A geometrical analysis of the data shows a potential structure for a neural code. When a 3-dimensional space is used to represent the responses, the responses to a single pattern appear to lie in a single plane regardless of luminance or duration, with the planes for different patterns frequently being separable. The equations for these planes describe a neural code.

PROJECT DESCRIPTION:Objectives:

The ability to perceive and recognize visual patterns requires the cooperative function of a sequentially connected system of cortical brain regions extending from primary visual cortex through inferior temporal cortex. The functionality of these regions arises from the properties of the single neurons in them. Thus, to understand how visual perception occurs, we must learn how information is encoded by the neurons in these successive stages of processing. One clear consequence of such understanding would be the ability to both predict the single neuronal signals to arbitrarily constructed stimuli, and to decode the responses to unknown stimuli. We have been recording single neurons in several regions of the visual pathway with the goal of developing a quantitative model of neuronal function. Such a model should simulate the activity of single visual system neurons in response to any arbitrary visual stimulus. Over the past several years we have made substantial progress toward this goal. With this knowledge and the knowledge of the anatomical organization gained from other work here, we can begin to explore how the cooperation of these individual building blocks gives rise to higher visual cognitive functions such as perception, attention, and memory.

Major findings:

A new approach to the study of single neurons was developed to carry out these studies. We conceptualize single neurons as communication channels that transmit information about visual patterns in their responses. This has allowed us to adapt and apply statistical, information theoretical, and signal processing techniques to neuronal responses.

To analyze a communication channel, a known set of signals is used as inputs, here visual stimuli, and then the responses are analyzed to find the representation of the input signal. To fully characterize the channel, the input set should cover the spectrum of all possible input signals that can be encountered. This requirement is usually met through use of a set of mathematically derived signals that can be added together to produce any arbitrary signal. Thus, we have used a set of orthogonal, two-dimensional, black and white patterns based on a complete set of Walsh-Hadamard functions as our visual input set. Each of these stimuli can be considered an independent, basic picture feature, and any picture can be made up as a sum of different ones. Using a general statistical technique, we identified the optimal set of temporal patterns, the principal components, that describe the responses.

To quantify the stimulus-response relation, we adapted and applied Shannon's information theory to the stimulus-response set. Our two-dimensional set of black and white Walsh patterns was defined as an input code, and the first three principal components were used to represent the responses as a temporally modulated output code. This analysis showed that neurons in both inferior temporal and primary visual cortex vary the strength and pattern of

their responses independently. The stimulus-related information is carried in three or more simultaneous and independent patterns of activity, i.e., information about different stimulus features is transformed into temporally modulated messages that are multiplexed onto the spike train. The amount of information carried by the temporal modulation is at least twice as great as that carried by the response strength alone.

From this we inferred that each neuron can be viewed as a small number (3-6) of simultaneously active spatial-to-temporal filters whose outputs are added or multiplexed together to form the response. In this multiplex-filter hypothesis, each filter gives rise to a temporally modulated message that corresponds to a different aspect of the visual pattern. The output of the filters, represented by the principal components, are then multiplexed onto the spike train. A model based on this multiplex-filter hypothesis has predicted the temporally modulated responses of striate cortex complex cells to arbitrarily constructed black and white patterns. Thus, this multidimensional description is a significant advance over less rich, unidimensional descriptions of neuronal responses, because neither static receptive field models nor unidimensional response strength measures can correctly predict the temporally modulated responses of a neuron.

According to a commonly held view of neuronal function, the strength of a neuron's response represents how closely the stimulus matches the receptive field's characteristics, e.g., orientation or color. Thus, if response strength were the only parameter a neuron could use to encode information, different stimulus features would be confounded by individual neurons. Using an informational analysis, we showed previously that information about different stimulus parameters is not confounded but is carried across the different parts of the multidimensional neuronal code. Furthermore, although information theory does not require that the neural code be interpretable in terms of these dimensions, we have been able to show that the neural code can in fact be so interpreted. When the responses were represented by the first three principal components and plotted in a space whose axes are these three principal components, the responses elicited by an individual Walsh pattern appeared to lie near a single plane irrespective of duration or luminance.

In any one neuron's principal component space, many of the planes representing the many Walsh patterns appeared easily differentiable. This geometrical structure demonstrates that the generation of neuronal responses obeys certain rules, which form an intrinsic temporal neural code for visual features. A response could be decoded to determine the stimulus pattern irrespective of duration or luminance if the plane into which the response falls could be ascertained. Information about duration and luminance would then be encoded relative to that plane. Since three points determine a plane, such a decoding scheme may require as few as three complementary neurons sharing related codes.

Now that we have found that temporal modulation of neuronal responses carries a substantial proportion of the stimulus-related information throughout the cortical visual system and have identified a potential set of rules that describe the neural code, we have tried to learn where the temporal modulation

first arises in the visual system. Presumably the responses of the retinal photoreceptors are proportional to the light falling on them. Therefore, the information carrying temporal modulation must arise between the photoreceptors and the visual cortex, and during the last year we have studied two of these stages: the lateral geniculate nucleus (LGN), and the pregeniculate fibers projecting from the retina, the ganglion cell fibers. Over the past 30 years, lateral geniculate neurons have been studied extensively, and their receptive fields are now generally described as having excitatory centers with inhibitory surrounds. However, their responses have never been examined for stimulus-dependent temporal modulation; it has simply been assumed that the stimulus-dependent information was encoded primarily if not exclusively in the response strength.

We recorded the responses of 12 parvocellular X-like units from the lateral geniculate nucleus of two fixating monkeys. The stimulus set consisted of 32 orthogonal black and white Walsh patterns, each presented at 7 different luminance combinations ($0.4 - 63 \text{ cd/m}^2$), and squares surrounded by annuli of the opposite contrast. The stimuli were based on either a 4×4 or 8×8 grid. Every stimulus was presented to each neuron a minimum of 5 times. The receptive fields were between 1 and 8 degrees eccentric to the fixation point. In consonance with all previous work, the luminance of the pixel stimulating the receptive field center governed whether the responses were excitatory or inhibitory. The strengths of the responses to squares with annuli were similar to those typically found in anaesthetized preparations. However, different stimuli elicited different temporal patterns in the responses. For example, transient responses could have one or two peaks, and sustained responses could have rising or falling slopes. Thus, even in the LGN, it appears that neuronal responses might carry a substantially richer description of the stimulus than is represented by the response strength alone.

During the same experiments we recorded from 5 fiber-like units, as judged by their low-amplitude, short duration ($< 0.25 \text{ ms}$) action potentials. These were located just above the lateral geniculate nucleus itself, where the optic tract spreads out over the nucleus. Thus, we hypothesize that these were ganglion cell fibers.

With these data we have been able to determine how much information about the stimulus was conveyed in the temporal modulation in these two locations. As before, responses were decomposed into their principal components, and the information conveyed in a spike count code was compared to the information conveyed in a temporal code made up of the first three principal components. For the LGN neurons, the information transmitted by the principal component code was 1.9 times the information transmitted by the spike count code ($0.99 \text{ vs } 0.54 \text{ bits, respectively}$). However, for the fiber-like units, the information in the temporal code was only 1.4 times the information in the response strength code ($0.99 \text{ vs } 0.72 \text{ bits, respectively}$), a ratio significantly smaller ($p < 0.02$) than that seen in the LGN.

Because our experiments are the first to systematically explore the whole extent of a visual pathway with similar methods, we can compare the temporal

modulation to response strength information ratios over the whole extent of the occipitotemporal visual pathway. These information ratios were 1.9 and 2.25 from striate cortex and inferior temporal cortex, respectively. Thus, the ratios from the fiber-like units are the lowest we have yet seen in the visual system, these ratios take a large jump in the LGN, and are largest in the pathway's final station. Because the total information transmitted by single neurons in all the areas we have studied is always about 1 bit, these comparisons show that the increased role of temporal modulation is due to the redistribution of information across response components rather than an increase in information carrying-capacity.

As described above, we had previously shown that striate cortical complex cells can be simulated by a model with parallel spatial-to-temporal filters. Since the results in the LGN were qualitatively similar, we tested a similar model based on the first 3 principal components of its temporally modulated neuronal responses because they accounted for as much as 75% of the variance in the responses. In this model each principal component was regarded as the output of an independent spatial-to-temporal filter. Each channel consisted of two components, a nonlinearity that compressed the luminances into a smaller range and a linear two-dimensional spatial filter. The model was then trained to respond to the Walsh patterns. After this training, the model was able to predict successfully the temporally modulated responses to "center-surround" annuli of different luminances.

We then developed a method to estimate the spatial distribution of the spatial-to-temporal filters. Unlike most previous estimates of the receptive field, our filters suggest that the receptive field is highly anisotropic. The first principal component is driven by a spatial filter that is positive but not radially symmetrical throughout its extent, whereas the second principal component is driven by a spatial filter that is dominated by a single bipolar pair of pixels. These estimates suggest that the classical center-surround mechanism may arise from the combined influences of two filters. The structures of the estimated filters suggest, in turn, that these neurons may encode a nonparametric estimate of the visual scene, possibly its luminance profile and the first derivative of the luminance profile.

Our results imply a new functional role for neurons in the visual system. Information about stimulus features is conveyed by individual neurons through multiple messages carried by a temporally modulated code. Like neurons in other parts of the visual system, lateral geniculate neurons simultaneously encode information about the luminance and luminance gradient of the scenes that fall within their receptive fields. Because the proportion of information transmitted by temporal modulation is substantially greater in LGN neurons than in ganglion cells, we hypothesize that one function of the LGN is to encode multiple stimulus features into a temporal code that keeps the information about different features separate.

Throughout the visual system, consolidation of local messages to determine global properties of images may be accomplished through compilation of many temporally encoded messages. Processing of information in visual areas may

consist not so much in altering the distribution of active elements but rather in transforming temporally modulated messages. We suggest that the hierarchical organization of feature abstraction posited for the multiple visual areas should be replaced by a progression of spatial-to-temporal filtering that changes the emphasis of the visual features but never confounds or ignores information.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Disorders of attention, perception, and memory accompany many psychiatric and neurological disorders. This project studies how information is encoded and transmitted. This knowledge will ultimately aid in the design of strategies both for more effective palliative treatment of cognitive deficits and for restitution of cognitive function.

PROPOSED COURSE OF RESEARCH:

Discovering that the responses of visual system neurons are multidimensional led to the discovery that information about multiple stimulus features may not be confounded by single neurons, a result with important, even revolutionary consequences. We now know that a substantial part of the temporal modulation arises after visual information has left the retina. Thus, the neural code may be a consequence of processing in areas under the influence of feedback, an hypothesis that will be tested experimentally.

Since we found evidence of a neural code and have seen a possible structure for it, we have been trying to delineate it. The properties of the code should give clues about the functions performed by the neurons. Also, the structures of the spatial filters seen in the lateral geniculate nucleus have already generated new ideas about the properties of encoded information there. Both these issues are being pursued.

New experiments are planned to make multiple, simultaneous single neuronal recordings. The simultaneously recorded responses will be related to each other through use of recent extensions to methods of signal identification, which should allow us to develop models that describe the roles of single neurons as components of larger networks relatively rapidly. These studies should yield a better understanding of the information transmission mechanisms used for cognitive functions such as pattern perception and recognition.

Our findings suggest a completely new conceptual framework in which to investigate neuronal function. One presumed reason for the huge number of single neurons has been the necessity to unconfound stimulus features. However, we propose that the simultaneous messages about different features can be used as tags, so that the messages that arise in different processing regions of the visual system can be reunited into a unified percept. This would provide the mechanism to build a whole perception across many processing regions. With the use of new computational equipment, this hypothesis can be explored both experimentally and theoretically.

PUBLICATIONS:

Richmond, B.J. and Sato, T. Enhancement of inferior temporal neurons during visual discrimination. J. Neurophysiol. 58: 1292-1306, 1987.

Richmond, B.J., Optican, L.M. and Gawne, T.J. Neurons use multiple messages encoded in temporally modulated spike trains to represent pictures. In J. Kulikowski (Ed.), Seeing Contour and Colour, Pergamon Press, Oxford, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02033-11 LN
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Functional Mapping of Sensory and Memory Systems		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: R.C. Saunders Senior Staff Fellow LN NIMH		
Others: M. Mishkin Chief LN NIMH J. Bachevalier Visiting Associate LN NIMH B. Agranoff Visiting Scientist Univ. of Mich. C. Kennedy Guest Researcher LCM NIMH L. Sokoloff Chief LCM NIMH		
COOPERATING UNITS (if any) Laboratory of Cerebral Metabolism, NIMH University of Michigan		
LAB/BRANCH Laboratory of Neuropsychology		
SECTION		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, MD 20205		
TOTAL MAN-YEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The cerebral areas related to vision in the rhesus monkey were identified by comparison of <u>metabolic activity</u> in visually stimulated versus visually deafferented cerebral hemispheres. The results allowed delineation of the visual-nonvisual borders of both an <u>occipitotemporal</u> and an <u>occipitoparietal visual pathway</u> and specification of their points of interaction with frontal, limbic, striatal, and diencephalic structures. In addition, it was found that, within the occipitotemporal pathway, the <u>forebrain commissures</u> contribute to the visual activation of area TE only. A <u>2-deoxyglucose double-label technique</u> has been tested and found to be capable of mapping the metabolic activity in the brain of a single monkey under two experimental conditions. It will be applied to the study of two different types of visual learning in the same subject. </p>		

PROJECT DESCRIPTION:

Metabolic mapping of the primate visual system

The extent of the two main cortical pathways known to be critical for higher order visual functions, the occipitotemporal and occipitoparietal pathways, were revealed in a comprehensive picture of the entire visual system at work, achieved by application of the [^{14}C] 2-deoxyglucose method. The method was applied while monkeys, restrained in a primate chair, either 1) passively viewed a high-contrast geometric pattern mounted on a surrounding rotating drum, or 2) actively performed a visual pattern discrimination task that required a response with the hand opposite the deafferented hemisphere. Earlier, the monkeys had received either an optic tract section combined with forebrain commissurotomy, and thus had one hemisphere visually deafferented, or had an optic tract section only, and thus had one hemisphere only partially deafferented. The comparison of local cerebral glucose utilization (LCGU) in the visually deafferented versus intact hemispheres within the same animal made it possible to identify and delineate the areas related to vision, whereas comparison of LCGU in the totally versus partially deafferented hemispheres across animals allowed assessment of the contribution to vision made by the forebrain commissures.

The deoxyglucose metabolic mapping procedure yielded the following picture. All cortical tissue caudal to the junction of the lunate and the intraparietal sulci is related to vision. Nonvisual tissue first appears in the superior parietal lobule (somatosensory) and at the beginning of the lateral fissure (auditory). In the parietal lobe, the upper border always remains within the intraparietal sulcus, about halfway down the upper bank caudally and closer to the fundus rostrally. The lower border moves from the lateral fissure and into the intraparietal sulcus rostrally. The rostral limit of visual tissue is located within the intraparietal sulcus, about 5mm behind its anterior tip. In the temporal lobe, the upper border always remains within the superior temporal sulcus, generally about halfway down the dorsal bank caudally but within the fundus rostrally. The lower border moves from the calcarine fissure to the hippocampal sulcus (where it continues midway along its length) and then turns laterally to enter the occipitotemporal sulcus and finally the fundus of the rhinal sulcus.

Subcortically in the temporal lobe, tissue related to vision occupies the lateral and lateral basal nuclei of the amygdala, posteroventral putamen, ventral claustrum, and the tail of the caudate nucleus. Visual tissue is also present in the anterior part of the head of the caudate nucleus, known to receive input from the visually related cortex of the inferior frontal convexity, and in both the body and the posterior portion of the head of the caudate nucleus, known to receive input from the visually related posterior parietal and prearcuate frontal cortices.

Comparisons of LCGU along the occipitotemporal pathway in the hemispheres with total and partial visual deafferentation revealed a significant difference

only in area TE, reflecting a contribution of the forebrain commissures to visual activation of this region alone. The likely explanation for the failure of commissural fibers to activate glucose metabolism in any part of the occipitotemporal pathway posterior to area TE is that the primary function of the commissural input to the posterior part of the pathway is to provide suppressive rather than excitatory influences on neural activity (see LN project MH 02036).

Metabolic mapping of the visual memory system

The two conditions of visual stimulation used for metabolic mapping of the visual system in the perceptual studies described above yielded virtually identical results. We are now attempting to map the further processing of visual information by requiring monkeys to perform a visual learning task. In addition, instead of using the 2-deoxyglucose [2DG] method employed in the perceptual studies, we will use the recently developed double-label technique, which will allow us to map brain metabolism related to two different types of learning in the same subject. The double-label procedure entails separate, sequential injections of [^{14}C]2DG and [^3H]2DG in an individual subject, with each injection immediately followed by a different experimental condition. Thus, the metabolic activity in the brain accompanying each experimental condition is indexed separately by the two radioactive labels.

We have recently applied this 2DG double-label technique to study the ocular dominance columns in striate cortex. Animals were trained to perform a visual discrimination task under two experimental conditions. In the first, during which [^{14}C]2DG was injected, one eye of the animal was covered while it performed the task. Twenty-five minutes later, [^3H]2DG was injected and neither eye was occluded while the animal performed. The first experimental period resulted in visualization of the ocular dominance columns in the striate cortex, whereas in the second period striate cortex was labelled uniformly. These preliminary results demonstrate that the double-label procedure works in the monkey.

To map the cognitive memory system, monkeys will be trained to perform the visual memory task, delayed nonmatching-to-sample (DNMS). This test has been used extensively to assess the effects on memory of cortical, limbic, and thalamic lesions (see LN Project MH 00478). To map the visual habit system for comparison, the same monkeys will also be trained on a series of visual discrimination problems. Performance on this task has been shown to be unaffected by the same limbic lesions that disable the cognitive memory system. After reaching a performance criterion of 90% correct responses in both tasks, the monkeys will be prepared with a forebrain commissurotomy combined with a unilateral amygdalectomy plus hippocampectomy. The monkeys will be retrained so that they perform at high levels even on the most difficult stages of the memory task. The double-label 2DG method will then be applied with the animals performing the memory task after the first label is injected and the discrimination task after the second label is injected. We

expect to be able to map the processing of visual information in both hemispheres beyond the areas seen in the visual perceptual studies. In the intact hemisphere (without the limbic system ablations) we expect to be able to visualize both the postulated limbo-thalamic memory system and, separately, the nonlimbic habit system, whereas in the hemisphere with the amygdalo-hippocampal ablations, we expect to visualize structures related to the habit system only.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

The 2-deoxyglucose method provides a unique method of relating neural structure and function, permitting for the first time both the visualization and quantification of local levels of metabolic activity simultaneously throughout the entire brain in animals studied either under normal conditions or following experimental intervention. The results continue to provide important insights into the role of various cerebral structures, both cortical and subcortical, in particular behaviors. Our initial studies contributed valuable information concerning the tissue occupied by the two cortical visual processing pathways, and we expect to obtain equally valuable information from our investigation of the two cortico-subcortical learning systems. The new double-label method should prove to be an especially powerful tool for this purpose.

PROPOSED COURSE OF RESEARCH:

Besides pursuing the metabolic mapping of learning and memory processes, we expect to apply the double-label 2-deoxyglucose method to the study of a variety of other behavioral processes in the monkey, including attention, emotion, and volition, for the purpose of identifying the various structures involved in these different behaviors and quantifying the degree of their participation.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02035-08 LN

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Anatomy of the Primate Visual System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L.G. Ungerleider Research Psychologist LN NIMH

Others: M. Mishkin Chief LN NIMH

R. Desimone Research Psychologist LN NIMH

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R.J. Tusa Asst. Professor Johns Hopkins Univ.

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Laboratory of Neuropsychology

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

4.75

PROFESSIONAL:

2.0

OTHER:

2.75

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To better understand the role of visual association cortex in perception and memory, we have examined the functional areas that comprise this cortex in the macaque and explored their interconnections by the use of neuroanatomical tracing techniques in combination with physiological recording of neural activity. Our results indicate that a multiplicity of separate visual areas lie beyond the striate cortex (V1) in the stream of information processing. These areas are organized into two divergent cortical pathways, each having V1 as the source of its initial input. One, an occipitotemporal pathway, enables the recognition of objects, while the other, an occipitoparietal pathway, mediates the appreciation of spatial relationships among objects as well as the visual guidance of movement. The areas along the occipitotemporal pathway (V1, V2, V3, V4, and TEO and TE of the inferior temporal cortex) appear to be organized as a serial hierarchy, in which each area processes both color and form. By contrast, the areas along the occipitoparietal pathway (V1, MT, and MT's projection zones in parietal cortex) process the direction of stimulus motion. Because a major component of this pathway extends anteriorly within the superior temporal sulcus, the neural mechanisms underlying visuospatial function may be more extensive than previously thought. Data from cerebral blood flow studies indicate the existence in humans, as in monkeys, of two distinct visual processing pathways, although there may be cross-species differences in their anatomical locations. To establish the links of both the occipitotemporal and occipitoparietal pathways with the motor system, we have explored the projections of visual association cortex to the striatum. The targets of these projections are the tail of the caudate nucleus and ventral putamen, which project in turn to the substantia nigra, pars reticulata (SNr) and the globus pallidus (GP). Because of the known inputs of the SNr and GP to the supplementary motor and premotor cortex via the thalamus, the striatum could be a link by which the visual system influences behavior.

PROJECT DESCRIPTION:

The long-term objective of this project is to understand the role of visual association cortex in perception and memory. To this end, we have been examining the multiple functional areas that comprise this cortex in the macaque and exploring the circuitry of their interconnections. So far, we have discovered that the primary visual area, striate cortex, is the source of two divergent corticocortical pathways: one, an occipitotemporal pathway, which enables the visual recognition of objects; the other, an occipitoparietal pathway, which mediates the appreciation of the spatial relationships among objects as well as the visual guidance of movements towards objects in space. Major questions to be answered in the future include: 1) How are the object and spatial information carried in these two separate pathways subsequently integrated anatomically to yield a unified visual percept? 2) What are the links of both pathways to affective, memory, and motor systems? 3) Can we identify the multiple visual areas in the human cortex that we have identified in the monkey?

METHODS EMPLOYED:

In these studies, we have used a variety of neuroanatomical tracing techniques (e.g., amino-acid autoradiography, horseradish peroxidase histochemistry, axonal transport of fluorescent dyes) in combination with electrophysiological recording of neural activity in anesthetized monkeys.

MAJOR FINDINGS:

1. An occipitotemporal pathway for object vision

We had previously found that the occipitotemporal pathway begins with the projection from the striate cortex, or V1, to the second and third visual areas, V2 and V3, which project in turn to area V4. These three prestriate areas are arranged in adjacent cortical belts that nearly surround the striate cortex, and, like the striate cortex, each belt contains a representation of the contralateral visual field. The major output of V4 is to a widespread region within the inferior temporal cortex. Within posterior inferior temporal cortex, or architectonic area TE0, label was found primarily after V4 injections involving the representation of the central visual field, whereas within anterior inferior temporal cortex, or architectonic area TE, label was found after injections of any part of V4. Thus, mainly central field representations in V4 project to TE0, while both central and peripheral field representations in V4 project to TE.

Physiological studies have shown that TE has no discernible visuotopic organization. Rather, neurons in TE have very large receptive fields that nearly always include the center of gaze and frequently cross the vertical meridian into the ipsilateral visual field. Thus, a single neuron in TE can "see" an object no matter where it occurs in the field, which is in keeping with the role this area plays in object recognition. Surprisingly little is

known about the properties of neurons within TEO. As a first step in studying these properties, we have begun to map TEO electrophysiologically. Thus far, we have found that TEO contains a crude representation of the upper quadrant of the contralateral visual field. Like V4, TEO forms an elongated dorsal-to-ventral band, and the representations of visual eccentricities seem to parallel those in V4. The representations of foveal and parafoveal visual fields are located on the inferior convexity of the hemisphere, adjacent to area TE, while the representation of the upper periphery lies on the ventral surface of the hemisphere, adjacent to unresponsive cortex. An especially high percentage of receptive fields recorded in TEO include the fovea, which is consistent with the major input this area receives from the central visual field representation of V4 and with the severe impairments in pattern perception that are seen following TEO lesions.

2. An occipitoparietal pathway for spatial vision

We had previously found that although area MT receives inputs from areas that participate in the occipitotemporal pathway (i.e. V1, V2, V3, and V4), its outputs appear to be mainly to areas located in the parietal lobe. One projection zone, area VIP, lies ventrally in the anterior two-thirds of the intraparietal sulcus, while two others, areas MST and FST, are located adjacent to area MT on the medial bank and floor, respectively, of the superior temporal sulcus (STS). Our experiments on the properties of neurons within the STS have demonstrated that, like neurons in MT, a majority of those in MST and a third in FST are directionally selective. Compared to neurons in MT, however, neurons in both MST and FST integrate motion information over progressively larger portions of the visual field and respond selectively to more complex types of visual motion. To identify additional components of this motion-analysis system, we have injected multiple anterograde and retrograde tracers into physiologically identified locations within MST and FST.

We have found that MST and FST send major projections to widespread regions of the posterior parietal cortex as well as to areas in the anterior STS, especially on its floor and medial bank. The projection zone within the latter region contains many cells with complex directional properties, such as sensitivity to rotation and optical flow. These results suggest that the cortical pathway for motion analysis, which begins with the projection of V1 to MT, splits into at least two components. One component includes regions of the posterior parietal cortex, whereas the other extends into the temporal lobe and includes several areas on the medial bank and floor of the STS. Thus, the neural mechanisms underlying visuospatial function may be far more extensive than previously thought. While it is known that lesions along the parietal component of this system cause impairment in spatial perception, smooth pursuit eye movements, and visually guided hand movements, the effects of lesions along the temporal component of this system remain to be explored.

Impairments in smooth pursuit eye movements following MST lesions are similar to those observed in patients with lesions at the junction of the parietal, temporal, and occipital lobes (PTO), suggesting that PTO in human cortex and MST in monkey cortex play similar roles in this function. Because impairments in patients often include the white matter, it is important to identify those fiber bundles in monkeys that interconnect the areas involved in smooth pursuit. We have found that the relevant white matter pathways consist of three different types of cortical fibers: 1) arcuate fibers, which course beneath the cortical mantle to interconnect striate cortex with MT, MT with MST, and MST with posterior parietal cortex; 2) the tapetum/major forceps, which is comprised of commissural fibers that pass through the splenium of the corpus callosum to interconnect MT and MST of one hemisphere with MT and MST in the opposite hemisphere; and 3) the internal sagittal stratum, which is the major subcortical fiber bundle projecting from MST to the dorsolateral and lateral pontine nuclei. Based on the effects of lesions on smooth pursuit, these corticocortical and corticosubcortical pathways can be divided into sensory, motor, and attentional/spatial systems. Evidence from clinical studies suggests that homologous systems exist in the human cerebrum.

3. Interactions of the occipitotemporal and occipitoparietal pathways

To determine how the object and spatial information carried by the occipitotemporal and occipitoparietal pathways are integrated to yield a unified percept, we have begun to investigate possible anatomical sites of interaction. Accordingly, we have made multiple injections of two different retrograde tracers, one into the lower bank of the intraparietal sulcus (following removal of the upper bank) and the other into the inferior temporal cortex, and then identified and compared the distributions of cells in extrastriate visual cortex projecting to these two destinations. Although cells projecting to temporal and parietal cortex were located mainly in different areas, two areas were found that contained cells projecting to both: V4 and the posterior bank and floor of the STS outside MT. In both V4 and STS, labeled cells projecting to the two destinations were intermingled, though the projection to parietal cortex was heavier from the peripheral than from the central field representation of V4. The laminar distribution of labeled cells suggests that V4 provides feedforward information to both temporal and parietal cortex, whereas zones within the STS provide both regions with feedback information. We are currently injecting the temporal and parietal cortex with different anterograde tracers to determine sites for convergence of information from these regions.

4. Mapping visual processing pathways in humans

We have recently undertaken a collaborative study with members of the Laboratory of Neurosciences (NIA) to investigate whether there are separate visual pathways in human cortex for processing object identity and spatial location. In this study, regional cerebral blood flow was measured with positron emission tomography (PET) as subjects performed both an object

identity and spatial location task. Areas activated more during the object than during the spatial task were located in occipitotemporal cortex, whereas areas activated in the spatial but not the object task were located in superior parietal cortex. These results demonstrate the existence in humans, as in monkeys, of two distinct visual processing pathways, although there appear to be cross-species differences in their anatomical locations.

5. Projections of visual association cortex to the striatum

To establish the links of both the occipitotemporal and occipitoparietal pathways with the motor system, we have been exploring the projections of visual association cortex to the striatum. Neurobehavioral studies in our laboratory suggest that these projections are the ones that mediate visual habits, whereas projections from visual cortex to the limbic system mediate visual memories. We have been particularly interested in determining the visual cortical areas that project to the striatum, how the projection fields relate to one another, how the cells of origin are organized, and to which structures the visual portions of the striatum project.

In these experiments, we placed multiple anterograde and retrograde tracers into physiologically identified portions of the striatum that receive input from visual cortex, namely, the tail and genu of the caudate nucleus and adjacent ventral putamen. After caudate injections, labeled cells were found both in a large continuous region of cortex topographically related to the site of injection, and in several non-contiguous cortical regions. After injections in the rostral tail, the continuously labeled region included rostral area TE and adjacent portions of TF, TH, TG, and, occasionally, area 35. After injections into the posterior tail and ventral genu, the labeled region shifted posteriorly to posterior TE, TF, and TEO and then into prestriate areas V4, MT, PO, and (sparsely) V3 and V2. As the injection site advanced into the dorsal genu and then to the caudal body, the labeled region shifted toward the parietal lobe, to area 7, to areas VIP and LIP in the intraparietal sulcus, and into area 5 and adjacent area 23. The non-contiguous areas labeled by nearly all injections included the principal sulcus/frontal eye field region, the anterior cingulate cortex, and the superior temporal polysensory area. Thus, whereas temporal, occipital, and parietal visual cortical areas project into the caudate largely according to proximity, multimodal cortical areas seem to have a wider distribution. Preliminary data on cortical projections to the ventral putamen indicate a similar pattern of results.

Because temporal and parietal cortex project to different parts of the caudate nucleus, this structure is probably not a site for convergence of temporal and parietal inputs (i.e. a site where the representation of objects and their locations in space could be combined). However, our results on the outputs of the tail and genu of the caudate nucleus and of the ventral putamen indicate that the targets of these structures could be sites where temporal and parietal inputs converge. These targets include the substantia nigra, pars

reticulata (SNr) and the internal subdivision of the globus pallidus (Gpi). Because of the known projections from the SNr and Gpi via the thalamus to the supplementary motor and premotor cortex, respectively, these cortical regions may represent further stations in the neural circuit underlying the formation of visual habits. Ultimately, we hope to delineate the entire wiring diagram of this circuit.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

An understanding of the basic mechanisms mediating normal visual perception and memory is the first step in the diagnosis, alleviation, and, ultimately, prevention of sensory, perceptual, and mnemonic disorders. To this end, we have been exploring projections out of the striate cortex to prestriate association areas. Our goal has been to trace the complex system of projections stepwise to the still higher-order visual areas located within the temporal and parietal lobes, areas critical for object vision and spatial vision, respectively. The combined use of axonal transport techniques and electrophysiological recording provides a powerful tool for tracing neural connections within these central visual pathways. In addition, the recent development of anatomical tracers that transport in fixed tissue, highly selective histological stains, and cerebral blood flow imaging techniques may give us the opportunity for the first time of identifying the same higher-order visual areas in the human brain that we have identified in the monkey.

PROPOSED COURSE OF RESEARCH:

Thus far, we have found that visual cortex in the monkey is organized into two divergent corticocortical pathways and that the projections of both pathways can be traced from the striate cortex through multiple prestriate areas to the still higher-order visual areas in the temporal and parietal lobes. Our recent studies suggest that both the temporal and parietal lobes also consist of multiple visual areas, and we will continue to investigate their organization. Since there are no direct connections between temporal and parietal cortex, we have begun to investigate how the object and spatial information carried in these two separate pathways are subsequently integrated anatomically. We also plan to continue our investigations of the links of both pathways to affective, memory, and motor systems by examining the projections of the multiple visual association areas to limbic structures, the prefrontal cortex, and the striatum. Finally, we will attempt to identify the multiple visual areas in the human cortex that have been differentiated in the monkey by: 1) using a combination of histological stains and degeneration techniques on human brain material with occipital lobe lesions; 2) injecting into identified areas of human brain newly developed anatomical tracers that transport in fixed tissue; and 3) using PET to measure cerebral blood flow in human cortex during the performance of a variety of visual discrimination and visual memory tasks.

PUBLICATIONS:

Segraves, M.A., Goldberg, M.E., Deng, S.-Y., Bruce, C.J., Ungerleider, L.G., and Mishkin, M. The role of striate cortex in the generation of eye movements in monkeys. J. Neurosci. 7: 3040-3058, 1987.

Tusa, R.J. and Ungerleider, L.G. Fiber pathways of cortical areas mediating smooth pursuit eye movements in monkeys. Annals Neurol. 23: 174-183, 1988.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02036-08 LN

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neural representations of visual stimuli in the extrastriate cortex

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OTHER:

1.25

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Disorders of perception, attention, and memory frequently accompany the major mental diseases. To begin to understand the neural mechanisms of these mental processes, we are recording the activity of neurons in the extrastriate cortex of monkeys engaged in tasks requiring visual discrimination, selective attention, and recognition memory. We found previously that selective attention gates visual processing by filtering unwanted information from the receptive fields of extrastriate neurons. Recently, we found that even the degree to which attended stimuli are processed by extrastriate neurons depends on "how much" attention or effort is devoted to them. When an animal is challenged by a difficult task, it appears to "rise to the occasion" by concentrating its attention, a correlate of which is sharpened selectivity of the neurons processing incoming sensory information. To identify the mechanisms by which cognitive state modulates cortical activity, we are examining both extrastriate neuronal activity and animal behavior in an attention-demanding task following lesions or chemical deactivation of structures that provide inputs to extrastriate cortex. These studies have identified two structures, each of which may play a different role in attentional modulation of cortical activity. One, the lateral pulvinar, appears to be critical for the ability to focus attention on a single stimulus and to ignore distracting stimuli. The other, the posterior parietal cortex, plays a more important role in spatial perception and the ability to shift attention from one location to another.

PROJECT DESCRIPTION:

This is a long-term project to understand both the neuronal basis of perception and memory in extrastriate cortex and the mechanisms by which these processes are influenced by cognitive factors such as selective attention. We focused our initial work on the basic sensory information coded by neurons in the extrastriate areas most directly involved in object recognition. Having identified several of the dimensions along which extrastriate neurons code objects, we have now turned to examining the dynamic operation of this cortical system in awake monkeys engaged in tasks requiring selective attention and memory.

Methods employed: Our most recent studies were carried out in awake monkeys, trained to hold their gaze steady while they performed a matching-to-sample task. In this task, the monkey held a bar while a stimulus appeared briefly at one retinal location followed shortly by a second, briefly presented stimulus at the same location. The monkey was rewarded for releasing the bar immediately if the two stimuli matched and for releasing the bar after a fixed delay if they did not match. At a second retinal location, two other, irrelevant stimuli were also presented on each trial, each concurrently with one of the stimuli used in the task. After a block of trials at the first location, a cue was given, and the previously irrelevant stimuli at the second location became the relevant (and, therefore, attended) stimuli for the matching task. Thus, identical sensory conditions were maintained across trials, but the locus of the animal's attention varied.

Experiments:

1. The influence of selective attention on neuronal processing in extrastriate cortex. Earlier in this project, we found that when a monkey attends to a stimulus within the receptive field of a neuron in either area V4 or area TE of extrastriate cortex, the processing of other, distracting, stimuli within the field is blocked. Thus, unwanted stimuli appear to be filtered out of the visual system as a result of selective attention, explaining why we perceive and remember only a small portion of the stimuli acting on our retinas at any given time. In subsequent experiments, we have found that even the degree to which attended stimuli are processed depends on "how much" attention or effort is devoted to them. For these studies, we recorded from neurons in extrastriate area V4 in monkeys trained on a visual discrimination task that had two levels of difficulty. Behavioral evidence indicated that the monkeys' discriminative abilities improved when the task was made more difficult. Correspondingly, neuronal responses to the stimuli became larger and more selective in the difficult task. A control experiment demonstrated that changes in general arousal could not account for the effects of task difficulty on neuronal responses. We concluded that when an animal is challenged by a difficult task, it "rises to the occasion" by concentrating its attention, a correlate of which is sharpened selectivity of the neurons processing incoming sensory information relevant to the task. Short reports on these findings have been published, and full-length reports are in

preparation. Having established that information processing in extrastriate cortex is controlled by cognitive state, our next step is to understand the neuronal basis of this control.

2. The source of attentional influences on neuronal responses. We found previously that the neuronal effects of spatially directed attention do not occur in either the primary visual cortex or area V2, so that whatever structure or structures gate extrastriate responses to attended stimuli must work at the level of V4 and beyond. Anatomical studies in our laboratory have identified at least four possible direct sources of modulating inputs to V4 and/or inferior temporal cortex, namely, portions of the pulvinar, posterior parietal cortex, prefrontal cortex, and limbic system. In addition, recent studies of the effects of unilateral dopamine depletion by Dr. Doris Doudet in LCM suggest that dopamine in the substantia nigra may play an indirect but very important role in attentional modulation of cortical activity. To determine the relative role of these structures in the control of attention, we have begun to test individually the effects of reversible deactivation of the lateral pulvinar or portions of the limbic system (using the GABA agonist muscimol), unilateral lesions of the dopaminergic system in the substantia nigra (using the neurotoxin MPTP), and removal of specific areas of the parietal and frontal cortex on the ability of monkeys to perform our attention task with distracting stimuli.

2A. The role of the pulvinar. Although our results are preliminary, they indicate that the lateral pulvinar plays an important role in the ability to focus attention on a single stimulus and ignore distractors. Unilateral deactivation of the lateral pulvinar did not appear to have any effect on visual discrimination performance when there was no distracting stimulus in the visual field. However, the monkey was severely impaired during deactivation of the pulvinar if a distracting stimulus was present, suggesting that the monkey could not ignore the distractor. As a control for the alternative possibility that the monkey was impaired in the ability to switch attention from one location to another, we measured its reaction time to detect a stimulus when its attention had previously been drawn elsewhere. These reaction times were normal, supporting the hypothesis that the lateral pulvinar is involved in the "focusing" aspect of attention rather than the switching aspect. We are now planning to record from extrastriate neurons during pulvinar deactivation to test whether the pulvinar is necessary for the attentional gating of extrastriate neuronal responses.

2B. The role of the posterior parietal cortex. Unlike the case for the pulvinar, a unilateral lesion of the posterior parietal cortex had no effect on the ability of a monkey to perform a form or color discrimination in the hemifield contralateral to the lesion in the presence of a distracting stimulus, suggesting that the posterior parietal cortex is not involved in focusing attention. However, the lesion did increase the time it took for the monkey to detect a stimulus when its attention had previously been drawn elsewhere, suggesting that the parietal cortex is important for the ability to disengage or switch attention, a possibility that is supported by the results of studies in humans. If the parietal cortex is involved in switching rather than focusing, we expect that posterior parietal lesions would have no effect

on the attentional gating of extrastriate neuronal responses once the monkey's attention was engaged on the stimulus. This prediction will now be tested in neuronal recording studies. The contrasting effects of pulvinar and posterior parietal cortex dysfunction support the notion that the mechanism underlying selective attention involves a number of different components, each of which may be functionally associated with a different neural circuit.

In addition to the effects of parietal lesions on switching attention, we unexpectedly discovered that the posterior parietal cortex plays an important role in judging the spatial orientation of an object in the visual field. Whereas the monkey's ability to discriminate forms or colors was normal in both visual hemifields, its ability to judge the orientation of a line was severely impaired in the hemifield contralateral to the lesion. We plan to pursue this surprising finding in future studies.

3. Neural mechanisms for recognition and associative memory. Although monkeys and man exhibit agnosia following damage to the cortex of the temporal lobe, there is remarkably little neurophysiological evidence that memories are actually stored in the cortex. To test this possibility, we have developed a system for presenting many complex visual and auditory stimuli in recognition and associative memory (or recall) tasks. Our initial recordings from neurons in area TE of the temporal lobe in monkeys performing a recognition task have been encouraging, in that they indicate that many cells do indeed respond differently to a visual stimulus depending on whether or not it has been presented previously to the monkey. Since we have so far only tested for these differences over very short time intervals, we do not yet know if these responses could form the basis for a long-term recognition memory. We have now trained a series of monkeys to perform a recognition memory task over longer time intervals and with multiple intervening stimuli, and we will examine the role of inferior temporal neurons in memory formation over the next year.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH

The results from our recording experiments in extrastriate cortex demonstrate that psychological factors such as selective attention and effort directly affect the cortical neurons that process incoming stimuli and store items in memory. If, during the future course of this project, we can determine exactly how these effects take place, we will be in a better position to understand, and, ultimately, treat the disorders of perception, attention, and memory that frequently characterize major mental diseases such as Alzheimer's disease, Parkinson's disease, affective disorders, and schizophrenia. Our recent results implicating the pulvinar in the ability to focus attention and the posterior parietal cortex in the ability to switch or disengage attention are important steps in this direction.

PROPOSED COURSE OF RESEARCH

A major thrust of our work over the next year will be to continue to track down the structure or structures that modulate cortical processing as a result of selective attention. Our general strategy will be to first identify likely

sources of the modulating input by testing the effects of lesions on the attentional capacities of monkeys. When a structure has been so identified, we will then record from its neurons while the animal performs our selective attention task, looking for neuronal responses related to the animal's switch of attention. Finally, we will attempt to model the system with computer-simulated neuronal networks.

PUBLICATIONS

Spitzer, H., Desimone, R., and Moran, J.: Increased attention enhances both behavioral and neuronal performance. Science 240: 338-340, 1988.

Desimone, R., Moran, J., and Spitzer, H.: Neural mechanisms of attention in extrastriate cortex of monkeys. In M. Arbib (Ed): Competition and Cooperation in Neural Nets 2, Springer-Verlag (New York), in press.

Desimone, R., and Ungerleider, L.: Neural mechanisms of perception in monkeys. In A. Damasio (Ed): Handbook of Neuropsychology, Elsevier (Amsterdam), in press.

Z01 MH 02037-07 LN

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functional Anatomy of the Somatosensory Cortex of the Monkey

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0.25

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unspaced type. Do not exceed the space provided.)

By use of anatomical markers to trace the connections between somatosensory cortical fields, we have identified two possible routes by which somatosensory information could reach limbic structures important for somatosensory memory. One route courses dorsally from the postcentral strip through posterior parietal cortex and has access to the limbic system via cingulate cortex. This pathway may be functionally analogous to the occipitoparietal pathway for spatial vision. The second route courses ventrally from the postcentral strip through SII and insular cortex to the amygdala and indirectly to the hippocampus through rhinal cortex. We now have strong evidence that this second pathway is analogous to the occipitotemporal pathway for object vision. First, by recording single- and multi-unit activity from the SII region in hemispheres that had received lesions of selected portions of the postcentral body representation, we have demonstrated loss of the corresponding representation from SII, indicating that at least this part of the ventrally directed somatosensory pathway is arranged as a cortical hierarchy. An unexpected finding in this study was that, following a postcentral lesion, the SII region undergoes major functional reorganization, in that the SII tissue vacated by the original representation does not remain silent but, instead, becomes occupied by representations of different body parts, providing evidence for a previously unrecognized degree of cortical plasticity in adult primates. Our neurobehavioral evidence indicates that bilateral insular lesions cause a severe tactile recognition deficit, consistent with the suggestion that insular cortex is a critical link in a parieto-insulo-limbic pathway for tactile recognition, and so occupies a position analogous to that of area TE in the occipito-temporo-limbic pathway for visual recognition. These comparisons suggest that sensory-limbic interaction through a hierarchically organized cortical pathway is a mechanism common to memory formation in all sensory modalities.

PROJECT DESCRIPTION:

Previous work from our laboratory has shown that the amgdala and hippocampus are critical for both visual and tactual memory. We have been trying to determine the most direct route by which tactual information could reach these limbic structures. In addition we have been trying to determine if there is a common cortical plan of organization across all of the sensory modalities for the processing, storage, and retrieval of sensory information.

Anatomical studies

Corticocortical connections. Anatomical studies have indicated that visual information is transmitted ventrally to the temporal lobe via a series of relays in prestriate areas. Specifically, V1 projects to V2 (area OB), V2 projects to areas V3 and V4 (both fields are part of area OA), and V4 projects to the inferior temporal areas TE and TEO. Area TE of inferior temporal cortex is the last cortical visual processing station in the sequence, and this area projects in turn to the amygdala (directly) and to the hippocampal formation (indirectly via perirhinal and entorhinal cortex). Also, area TE is totally dependent upon input from the more posterior areas in the chain for visual activation of its neurons. This pathway, which remains modality specific throughout its neocortical extent, has been shown to be important for the visual recognition and identification of objects.

By contrast, a second cortical visual pathway is thought to be important for visuospatial perception. This is a dorsally directed cortical pathway that begins in V1, passes through prestriate visual areas, courses through posterior parietal cortex, and then projects to the cingulate cortex before finally reaching the amygdala and hippocampus.

To determine whether analogous pathways for stimulus quality and spatial perception might be present in all of the sensory systems, we turned to the somatosensory system, since more is known about its cortical organization than about that of any modality except vision. Our experiments indicated that the fields comprising postcentral somatosensory cortex (areas 3a, 3b, 1, and 2) are richly interconnected with each other and that these connections link corresponding representations in the different fields. In addition these fields have further connections with areas outside the postcentral cortex. On the basis of the laminar patterns of these various connections, each was designated as a forward projection (layer III to layer IV) or as a backward projection (layer V to layer I) by analogy to similar designations in the visual system. From this analysis, we were able to identify two major pathways for the flow of somatic information out of postcentral cortex.

The first cortical pathway is directed ventrally and begins with the primary cortical receiving area for tactual information, area 3b. Area 3b projects forward to area 1 and less densely to area 2. The densest cortical projection from each of these areas (3b, 1, and 2), however, is to layer IV of SII cortex. SII then projects in a forward manner to the granular and dysgranular fields of insular cortex, and, finally, the pathway proceeds from the fields

of the insula to the amygdala and indirectly to the hippocampus through perirhinal cortex. As indicated below, we have been slowly accumulating additional physiological and behavioral evidence that this pathway in the somatosensory system is analogous to the ventrally directed pathway in the visual system and consequently is important for the tactile identification of objects.

The second pathway is a dorsally projecting one, again with area 3b projecting forward to area 1, and less densely to area 2. Area 1 in turn projects forward to area 2 and to a specialized cutaneous portion of area 5. Area 5 in turn projects to area 7b of posterior parietal cortex. Each of the above areas projects with a backward connection upon the cortical area from which it receives a forward connection. This dorsally projecting somatosensory pathway may be analogous to the occipitoparietal pathway in vision and thus could be important for spatial perception in somesthesia.

Electrophysiological studies

Receptive fields of SII neurons following ablation of postcentral cortex. It had previously been assumed that the primary thalamic nucleus for tactual information (the ventroposterior nucleus, or VP) supplied the major activating input for both postcentral cortex and SII. However, our anatomical studies summarized above suggested instead that SII cortex may be receiving its somatosensory input from postcentral cortex, rather than directly from the ventroposterior nucleus of the thalamus. To examine this possibility, we recorded single- and multi-unit activity from the SII region in 10 hemispheres of 6 macaques (4 *Macaca mulatta* and 2 *Macaca fascicularis*) anesthetized with a mixture of halothane and nitrous oxide. The electrode penetrations were placed 0.5-1.0 mm apart in a rectangular grid across the entire extent of SII, and neuronal responses were sampled at 200um intervals through the depth of this cortex. The receptive fields of the neurons at the recording sites were determined by applying tactile stimulation at different locations on the contralateral body surface. Of the 10 hemispheres studied, 5 were intact and 5 had received lesions 6-8 weeks earlier of selected portions of the body representations in the postcentral strip.

In the intact hemispheres, receptive fields of neurons in SII were readily found for tactile stimulation of all contralateral body parts, with the majority of the fields representing loci on the glabrous and hairy surfaces of the hand. By contrast, in recording sites through SII of hemispheres in which the postcentral hand representations had been removed, no receptive fields could be found for tactile stimulation of the glabrous surface of the hand, and only a few were found that included the hand's hairy surface. Yet there was no difficulty in recording responses in the experimental hemispheres to stimulation of all other body parts, indicating that the near absence of a hand representation in SII was not due simply to a general postsurgical depression of the SII cortex. The functional dependency on postcentral cortex that was demonstrated for the SII representation of the hand held also for the SII representations of other body parts. For example, in recording sites

distributed through the SII region in a case with a postcentral removal that spared only the hand representation, all of the receptive fields found were confined to the hand. Similarly, in recording sites through SII in a case with a total removal of the postcentral strip, no neuronal activation was observed from tactile stimulation of any body part. In short, the elimination of any body-part representation in the postcentral cortex eliminated it also in SII. The results thus support the proposal derived from our anatomical studies that SII depends on the postcentral strip for its somatic activation and thus could well occupy an intermediate position between the postcentral cortex and the insula in a sequential cortico-limbic pathway for touch. Of the four areas that comprise postcentral somatosensory cortex, two (areas 3b and 1) process cutaneous inputs predominantly, while the other two (areas 3a and 2) process mainly "deep" somatic inputs. We have found that, following selective removal of the 3b and 1 hand representations, the SII hand representation is still present, but activation of its neurons requires a substantially higher-than-normal amplitude of somatic stimulation. We used a pressure aesthesiometer to quantify the force required to elicit a consistent neural response from recording sites in different representation in SII 6-8 weeks after the selective ablations. Whereas a stimulation force of less than 0.5 gr was adequate to produce a response at a majority of recording sites outside of the SII hand region, a stimulation force of more than 2 gr was necessary to evoke a response at sites within it, presumably because of the need to activate "deep" somatic inputs from the hand. These findings suggest that modality-specific information is relayed from the postcentral strip to SII along parallel channels, with cutaneous inputs being transmitted via areas 3b and 1 and "deep" inputs via areas 3a and 2.

Postoperative cortical plasticity. Another, unexpected result from the foregoing electrophysiological work was the finding that the SII region undergoes major functional reorganization following removal of portions of postcentral cortex. As indicated above, removal of all the representations of a body part in postcentral cortex (i.e. including its maps in 3a, 3b, 1, and 2) results in the failure to record somatically driven responses in the representation of the corresponding body part in SII. Interestingly, the SII tissue in question does not remain silent; instead, representations of different body parts in the adjacent portions of SII expand to occupy the partially deafferented cortical zone. For example, following a lesion of the postcentral representation of the hand, there is a greater probability of recording responses in SII to stimulation of the foot. Indeed, the areal extent of the foot representation increases to occupy most of the former hand region (a distance of 5 or more millimeters of cortex). These findings provide evidence for a previously unrecognized degree of cortical plasticity in adult primates. The results thus require major revisions of current theories, which tend to confer static properties on cortical maps.

Receptive fields of insular neurons. In another recording project, we are mapping the somatically responsive portions of the insular cortex, which our anatomy shows receives a dense input from SII, in an attempt to determine the somatotopic organization and response properties of cells in this region.

Because anesthetics are known to depress higher order sensory cortical areas such as the insula, this study must be performed in awake responding monkeys trained to sit in a primate chair and to allow gentle tactile stimulation of their bodies. The preliminary results indicate that receptive fields for insular neurons are typically very large, usually bilateral, and are modality specific. Our recordings have also revealed that there is at most only a rough somatotopic organization within the granular insular cortex, with the face and intraoral regions being represented rostrally, and the rest of the body being represented more caudally. These receptive-field properties are clearly analogous to those of neurons in visual area TE, in that they too are modality-specific, are large and bilateral, and have poor topographic organization. Our data are thus consistent with the notion that insular cortex serves as a final link in a somatosensory-limbic pathway just as area TE does in the visuo-limbic pathway.

Metabolic mapping studies

To further test the possibility that corticocortical projections terminating in layer IV carry sensory information forward from one field to another, we have combined electrophysiological, anatomical, and metabolic mapping techniques. Area 3b projects to layer IV of area 1 and area 1 projects to layer IV of area 2. We therefore predicted that if we were to identify electrophysiologically a small region of cortex in areas 3b or 1 activated by a well defined, restricted peripheral tactual stimulus and injected an anterogradely transported marker into that site, the distribution of terminal label in layer IV of areas 1 or 2 would coincide there with the metabolically activated region.

We first located cell clusters in area 3b or in area 1 that responded to precisely defined tactual stimuli applied to the fingers or toes. We then iontophoretically injected WGA-HRP into those sites so as to produce injection sites that were 0.5-1mm in diameter, corresponding to the size of the cortical region that is activated by restricted peripheral stimuli. Two days later, we injected the animal with [^{14}C]2-deoxyglucose and stimulated the peripheral receptive fields that had been defined earlier with a mechanical stimulator that allowed us to reproduce exactly the parameters of stimulation that had best activated the neurons from which we had previously recorded.

Comparison of the two types of label demonstrated that within areas 1 and 2 the patches of anterogradely labeled HRP terminals were precisely aligned with the stimulus evoked 2-DG patches. Control experiments, in which the stimulus site used for the metabolic activation did not match the site injected during the recording session, yielded a nonalignment of the two types of label.

Neurobehavioral studies

We have been using behavioral techniques to examine the effects of removing the insula on tactile object recognition and have postoperative results from four animals indicating that bilateral insular lesions cause a tactile

recognition deficit. This preliminary finding is consistent with the suggestion that insular cortex acts as a final link in a parieto-insulo-limbic pathway for somesthesia, analogous to the occipito-temporo-limbic pathway previously described for the visual system. This preliminary result is particularly exciting, for, if it is upheld, the project has the potential of revealing a mode of sensory-limbic interaction that is common to memory formation in all sensory modalities.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

The data from these projects are providing us with the first comprehensive view of the entire somatosensory system as well as of its connections with the limbic memory system. Furthermore, the studies have suggested remarkable parallels between the organization of the somatosensory and visual systems and imply that similar mechanisms of perception and memory may operate within both. These projects are yielding fundamental insights into how the cerebral cortex processes and stores sensory information by uncovering mechanisms that may well be common to all sensory modalities. Finally, we have demonstrated a previously unrecognized degree of post-injury cortical plasticity in the adult macaque with the exciting discovery that SII cortex undergoes extensive functional reorganization following damage to primary sensory cortex.

PROPOSED COURSE OF RESEARCH:

More neurobiological and behavioral evidence is required to assess our hypothesis that the ventrally directed cortical pathway in the somatosensory system is analogous to the ventrally directed one in the visual system, and the necessary studies will be continued. In addition, we plan to begin research projects designed to determine if the dorsally directed somatic cortical pathway has a role in tactual spatial perception by analogy to the role of the dorsal visual pathway in visual spatial perception.

PUBLICATIONS:

Kaas, J.H. and Pons, T.P. The somatosensory system of primates. In H.P. Steklis (Ed.): Comparative Primate Biology, Vol. 4, Alan Liss, New York, in press.

Pons, T.P., Garraghty, P.E., and Mishkin, M. Lesion-induced plasticity in the second somatosensory cortex of adult macaques. Proc. Nat. Acad. Sci. 85: 5279-5281, 1988.

Pons, T.P., Garraghty, P.E., and Mishkin, M. Plasticity in nonprimary somatosensory cortex of adult macaques. In H. Flohr (Ed.): Post-lesion neural plasticity, Springer-Verlag, in press.

Pons, T.P. Representation of form in the somatosensory system. TINS, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02038-06 LN

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ontogenetic Development of Cognitive Memory and Habit Formation

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TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

0.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Cognitive memory and habit formation are two qualitatively different learning processes based on separate neural systems, a cortico-limbic and a cortico-nonlimbic system, respectively. To see how emotional and social behavior develop in animals whose infantile global amnesia might persist from infancy through adulthood, we have prepared monkeys with neonatal limbic lesions and followed their behavioral development. Animals with neonatal removal of cortical area TE, a higher-order visual station linked to both learning systems, serve as controls. Assessment of the effects of these neonatal lesions on the early development of habits and memories points to greater compensatory potential after neonatal cortical than after neonatal limbic removals. In addition, the long-term effects of these early lesions indicate that compensatory mechanisms operate early to promote permanent recovery from neonatal temporal cortical but not from neonatal limbic lesions. These findings suggest that association areas of the cortex are immature at birth, and may thus possess greater plasticity than limbic structures. Direct evidence of neocortical immaturity in the macaque has been provided by our neurobiological studies on opiateergic and cholinergic receptor distribution and on metabolic activity. Data on both normal and operated infants suggest that development of the nonlimbic habit system is sexually dimorphic, and that this is due to the high testosterone levels present in male infants before and shortly after birth. Interestingly, early damage to the limbic memory system produces later socio-emotional abnormalities. The developmental time course and the nature of these disturbances resemble those seen in autistic children. Finally, the studies in young adult monkeys together with those in normal aged animals are providing the anatomical and chemical basis for understanding the memory disorders in humans that accompany cerebrovascular and other cerebral accidents and diseases as well as the gradual decline in memory ability that occurs with normal aging.

PROJECT DESCRIPTION:

Experiment 1

Findings from studies of the effects of lesions in adult monkeys suggest that cognitive memory and habit formation are qualitatively different retention processes based on separate neural mechanisms. The cognitive memory system, which serves both recognition and associative memory, utilizes a cortico-limbo-diencephalic circuit. By contrast, the habit system, which mediates the formation of stimulus-response connections, probably depends in large part on a cortico-striatal system. Our recent studies of behavioral development in infant monkeys have suggested that these two systems are developmentally dissociable, in that the nonlimbic habit system appears to mature considerably earlier than the limbic memory system. On the evidence that the limbic memory system is essentially nonfunctional in infants, we have prepared monkeys with neonatal removal of this system in an attempt to see how emotional and social behavior develop in animals whose amnesia might persist from infancy through adulthood.

To date, eight infant rhesus monkeys received damage to the limbic system (i.e. amygdalo-hippocampal complex) and eight others, which served as control animals, received damage to the anterior part of inferior temporal cortex (i.e. cytoarchitectonic area TE, a higher-order station of the visual system). These monkeys were age-matched with thirteen normal animals and have already undergone some testing for social behavior and learning abilities. We are currently following the behavior of these animals from birth through early adulthood in order to assess (1) the maturation of cognitive memory and habit formation and (2) the development of emotional and social behaviors.

Maturation of cognitive memory and habit formation in early infancy. At ten months of age, the infants with limbic lesions are severely impaired in cognitive memory, whereas those with area TE lesions show significant functional sparing of this ability (compared to the animals given TE lesions as adults). The results point to greater compensatory potential after neonatal cortical than after neonatal limbic removals, and are consistent with the notion that association areas of the cortex are less mature at birth, and may thus possess greater plasticity, than limbic structures. (Direct evidence of neocortical immaturity in the infant macaque is provided in Experiments 3 and 4.) The findings suggest that the reduced recognition ability in infant monkeys and perhaps, by extension, infantile global amnesia in this species are due more to slow maturation of the cortical association areas than to neonatal immaturity of the limbic system.

To investigate whether the severe impairment in cognitive memory in infants with the limbic lesions is due, as it is in adults, to the combined damage of the amygdala and the hippocampus and not to the damage of either limbic structure alone, we have prepared new groups of infant monkeys with damage to either the amygdala or the hippocampus only. These monkeys were age-matched with normal infants and were tested in exactly the same way as the infants

with combined limbic lesions. Whereas both amygdalar and hippocampal lesions in adulthood yielded a mild memory loss, only the neonatal amygdalar lesions produced such a deficit. The neonatal hippocampal lesions, by contrast, left this ability intact.

Some of the first infants to receive neonatal lesions are now four years old and have been retested to assess the long-term effects of these early lesions on formation of both noncognitive habit and cognitive memory. Thus far, 3 animals with neonatal area TE lesions, 3 others with neonatal limbic lesions, and 3 normal controls have been retested on object discrimination learning with 24-hr intertrial intervals to measure habit formation, and on delayed nonmatching-to-sample with trial unique objects to measure memory formation. Their performance was compared to that of four-year-olds that received the same lesions in adulthood. When tested at 4 years of age, the monkeys with neonatal damage to area TE exhibited significant sparing of both habit and memory formation of the same magnitude as that found earlier when the animals were 3 and 12 months of age. By contrast, when tested at 4 years of age, those with both neonatal and adult limbic lesions were unimpaired in habit formation but severely impaired in memory formation. Apparently, compensatory mechanisms operate early to promote permanent recovery from neonatal temporal cortical lesions but not from neonatal limbic lesions.

Development of emotional and social behaviors in early infancy. The slowly accumulating data of this long-term neurobehavioral study suggest that, compared to intact infant monkeys and those with neonatal cortical lesions, monkeys with neonatal limbic lesions show numerous socio-emotional abnormalities. At 2 months only, the infants with limbic lesions had more temper tantrums when first placed in a novel play cage, showed more passive behavior, and manipulated objects less than the controls. On the other hand, they did not display the more striking pathology they exhibited at 6 months, namely, lack of social contact, extreme submissiveness including active withdrawal, and gross motor stereotypies. The developmental time course and the nature of these disturbances in animals with limbic lesions resemble those seen in autism. Indeed, our results, combined with Kemper's recent report of neuropathology in the amygdala, hippocampus, and cerebellum of three autistic subjects, support the view that early dysfunction of the limbic system is one cause of infantile autism. Although amygdalar damage by itself is associated with changes in emotional and social behavior in adult monkeys, the socio-emotional abnormalities we observed in the developing infants with combined amygdalo-hippocampal lesions was not attributable to amygdalar damage alone. Rather, the full-fledged syndrome described above was fractionated by partial limbic lesions. Specifically, whereas amygdalar lesions in infants produce the same behavioral abnormalities that they do in adults, neonatal hippocampal removals also induce socio-emotional abnormalities, a finding that has not been reported before at any age. Together these experiments are providing evidence that the amygdala and hippocampus are each components not only of a limbo-thalamic system serving cognitive functions but also of a limbo-hypothalamic system serving emotional functions. Though much more

testing over a much longer time course is necessary, it is becoming clear that the same neonatal damage that leads to a severe cognitive memory disorder can have extremely serious consequences for personality and social development, in part perhaps because the cognitive memory disorder is present from infancy onward, but also because of the direct effect of the limbic lesions on mechanisms of emotionality.

To compare the socio-emotional abnormalities in the neonatally operated animals with those observed in autistic patients, we will retest the animals as they reach adulthood and analyze in detail their individual and social behavior for characteristic signs of autism, such as disinterest in initiating play when placed in a group, lack of separation anxiety, fearful responses to novelty and environmental complexity, and unusual food preferences.

One way to measure separation anxiety in monkeys is to record "isolation" calls. These calls serve to reestablish contact between a mother and her offspring, as well as between peers raised together. Cerebral ablation studies in squirrel monkeys have demonstrated that these calls can be eliminated by lesions of the cingulo-thalamo-limbic system. In collaboration with Dr. John D. Newman of the Laboratory of Comparative Ethology (NICHD), we have begun to record "isolation" calls in normal infants and infants that received either neonatal amygdalectomy or neonatal hippocampectomy. Our findings indicate significant differences in the acoustic structure of the calls from animals with amygdalar ablations, reflecting a lack of separation anxiety in animals with neonatal amygdalectomy. More surprisingly, animals with hippocampal lesions also exhibited some acoustic abnormalities, although these abnormalities were less clear and more variable. These findings support the view that the amygdala plays an important role in the development of normal affective vocal responses, and suggest for the first time that the hippocampus may also be involved in the development of this aspect of primate vocal behavior.

Experiment 2

The cortico-nonlimbic habit system becomes functional in female infants earlier than in males. The evidence for this sexual dimorphism comes from the findings that female infants learn visual discrimination habits faster than males, and neonatal ablation of area TE impairs learning of female but not of male infants. These two functional differences, which are apparent only when the monkeys are less than about six-months-old, indicate that area TE or its striatal target matures faster in females than in males. In a recent neuroendocrinological study, we have further demonstrated that the high levels of testosterone present in male infants before and shortly after birth are probably responsible for this sexual dimorphism, since a significant correlation appeared in male infants between their testosterone levels and learning scores (the higher the level the poorer the scores), and also because orchietomy in male infants actually speeded their rate of habit formation to equal that of females. We extended this experiment by adding a group of

infant females that were ovariectomized at birth and which then received either testosterone propionate (TP) or dihydrotestosterone (DHT), the two active forms of testosterone. Although the TP treatment proved to be ineffective, the DHT treatment significantly slowed the rate of habit formation to that seen in normal males. These new findings provide the first direct experimental evidence that gonadal hormones in the primate fetus influence the maturation of a telencephalic system important for learning.

Experiment 3

The 2-deoxyglucose method was applied postoperatively to a series of infant monkeys that had received unilateral optic tract section combined with forebrain commissurotomy at 1 day, 1 week, and 1, 2, 3, 4, and 6 months of age. In all visual cortical areas of the intact hemisphere, LCGU was lowest in the youngest subjects, peaked at 4 months, and then declined in the 6-month-old subject to levels found in adults. Interestingly, for area TE, differences in rates of glucose utilization between the "seeing" and "blind" hemispheres were small up to 3 months of age and reached adult levels only at about 6 months. This functional immaturity of area TE in early infancy is consistent with behavioral data indicating that adult levels of visual object recognition probably do not develop until about this time and that neonatal removal of inferior temporal cortical area TE produces significant sparing of this function (see Experiment 1), probably because of the immaturity of visual association areas (see Experiment 5).

Experiment 4

Direct evidence that limbic and cortical systems differ in rate of development has been provided by our developmental neurobehavioral studies. In previous experiments, we found that the distribution of opiate and muscarinic receptors in the macaque brain is adult-like at birth in limbic and striatal structures but is not yet fully developed in neocortical areas. Thus, like many other aspects of neocortical maturation, opiate and cholinergic mechanisms continue to develop postnatally. We are continuing to follow the developmental course of these mechanisms by examining them in infants of different ages.

Experiment 5

Visual recognition in adult monkeys is critically dependent on a neural system that includes both the inferior temporal cortical area TE and limbic structures. As described in Experiment 1, however, infant monkeys given lesions of area TE show significant sparing of visual recognition ability. Through the use of behavioral, anatomical, and metabolic mapping techniques, we are testing two different hypotheses that could account for this sparing of function, namely, (1) that afferents from other visual cortical areas innervate the limbic tissue that was deafferented by the area TE lesions, and (2) subcortico-limbic interaction substitutes for the absent cortico-limbic

interaction. Our first attempt was to investigate whether other visual cortical areas (areas PG, TF, TEO, and STP) connected with limbic structures but not normally necessary for object recognition become necessary if area TE is removed in infancy. Thus, areas PG, TF, TEO, and STP were ablated in a 2-year-old rhesus monkey that had undergone area TE removal neonatally and had shown sustained recovery of object recognition. Following the secondary removals, the monkey demonstrated significant impairment in visual recognition. Apparently, one or more areas removed in the secondary lesion had assumed a critical role in object recognition as a result of the neonatal TE lesion. Presumably such compensation is possible because, as demonstrated in Experiments 3 and 4, visual association areas are not yet fully mature at birth and so still possess significant functional plasticity.

Experiment 6

In collaboration with Dr. William Overman of the Department of Psychology at the University of North Carolina at Wilmington, we have begun to assess visual recognition in human infants using the same task we used in infant monkeys, namely, delayed nonmatching-to-sample with trial-unique stimuli. Human infants at different ages are being tested in exactly the same way as the infant monkeys and the preliminary results indicate that whereas infant females reach adult levels of performance around 20 months of age, males do not do so until 26 months of age. Interestingly, the developmental time course of this ability in humans parallels that already described for infant monkeys (see Experiment 1).

Experiment 7

In collaboration with investigators from The Johns Hopkins University School of Medicine, we have begun an assessment of the decline of learning and memory in aged monkeys. We found impairments in a wide variety of learning and memory tasks (delayed nonmatching-to-sample, direct delayed response, concurrent object discriminations, route following, motor skills, emotional responsiveness, food preference, and response latency), suggesting that there is widespread cerebral dysfunction in aged rhesus monkeys, probably due to the vulnerability of multiple neural systems to increasing age. At the same time, these learning and memory impairments vary widely from one aged animal to another within a given task, and there is no correlation in degree of impairment for a given aged animal across tasks, suggesting that the distribution and density of age-related neuropathological changes is likely to vary considerably from animal to animal. This possibility will be investigated directly through postmortem localization of neuritic plaques and depletion of cholinergic and other neurotransmitters. Thus, the use of the aged nonhuman primate as a model for human aging will provide important information regarding the relationship between age-related cognitive changes and pathological alterations in the brain.

Experiment 8

The mnemonic and neuropathologic effects of blockage of the posterior cerebral arteries (PCA), a cerebrovascular accident that can lead to global anterograde amnesia in humans, has been investigated by occluding these arteries bilaterally in several monkeys. The monkeys' memory was then evaluated using a visual recognition task, after which the extent of their ischemic infarcts was assessed. The latter showed substantial individual variation, ranging from almost no damage in one case to massive unilateral injury of both the ventromedial occipitotemporal cortex and hippocampal formation in another. In the remaining cases, however, the infarcts all fell within a narrow range, being confined almost entirely to the hippocampal formation and parahippocampal gyrus, and then only to restricted portions of these structures. These cases with bilateral infarctions, involving between 20 and 55 percent of the hippocampal formation, showed substantial recognition impairment, with scores averaging 20 percent below those of normal controls. The only subfields of the hippocampus damaged in common in these cases were CA1 and CA2. Paradoxically, the memory loss found in these animals with only partial bilateral hippocampal damage was significantly greater than that found in animals with total bilateral ablation of the hippocampal formation, whose scores averaged only 10 percent below those of normal controls. This paradoxical finding may help explain the severe memory deficit found in human patients with only partial damage to the hippocampus resulting from hypoxic episodes.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Developmental studies of the effects of early brain damage are of great importance for the assessment and understanding of those errors of central nervous system maturation that cause children to become autistic, dyslexic, learning disabled, or mentally retarded. This project will provide the first comprehensive evaluation of the social and cognitive development of monkeys suffering from an amnesia induced by limbic lesions early in infancy as compared to those rendered amnesic in adulthood, i.e. after memories have been formed and consolidated in cerebral tissue outside the limbic system. In addition, comparison of the effects of early and late cortical and subcortical lesions will help answer whether or not compensatory mechanisms always operate to promote recovery from early brain injury. Our preliminary results suggest otherwise. Our neurobiological studies indicate how brain maturation normally progresses postnatally and our neuroendocrinological studies demonstrate how the perinatal hormonal environment may influence brain maturation and, consequently, the development of cognitive functions. Finally, the studies in young adult monkeys together with those in normal aged animals are providing the anatomical and chemical basis for understanding the memory disorders in humans that accompany cerebrovascular and other cerebral accidents and diseases as well as normal aging.

PROPOSED COURSE OF RESEARCH:

Our goal is to continue examination of the effects of neonatal limbic lesions on social and emotional behavior as well as on cognitive memory and habit formation at several periods throughout development from infancy to adulthood in order to test whether such a preparation does indeed provide an animal model of childhood autism. We shall also pursue studies to determine how recognition memory measured by preferential viewing differs from recognition memory measured by problem solving and compare the developmental time-course of this ability in infant monkeys to that seen in human infants. This will help determine which capacities of the memory system appear late in ontogenetic development and, by implication, whether the phenomenon of infantile amnesia might be due to the absence of a fully functional cognitive memory system in early childhood. We shall continue our attempts to follow the development of neurochemical receptors in infant monkeys. In addition, new experiments have been initiated to study the neural mechanisms by which visual object recognition can develop in the absence of area TE, the highest-order area of the visual pathway. Finally, we will pursue our longitudinal study on memory decline in normal aging.

PUBLICATIONS:

Presty, S.K., Bachevalier, J., Walker, L.C., Struble, R.C., Price, D.L., Mishkin, M., and Cork, L.C. Age differences in recognition memory of the rhesus monkey (Macaca mulatta). Neurobiol. Aging 8: 435-440, 1987

Bachevalier, J. and Mishkin, M. Mnemonic and neuropathological effects of occluding the posterior cerebral artery in Macaca mulatta. Neuropsychologia, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02039-06 LN

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less, Title must fit on one line between the borders.)

Pharmacology of Cognitive Memory and Habit Formation

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TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unproved type. Do not exceed the space provided.)

Evidence from patients with Alzheimer's disease suggest that the basal forebrain cholinergic system plays an important role in memory. In support of this proposal, we have found impaired visual recognition memory in macaques with lesions to the major nuclei of this system. We have found further that recognition memory in normal monkeys can be improved by the cholinesterase inhibitor physostigmine and impaired by the cholinergic muscarinic-receptor blocker scopolamine. Another form of retention, spatial memory, has also been found to be impaired by scopolamine, although it is more resistant to such an effect than is recognition memory. In addition, our results indicate that scopolamine acts at a very early stage, suggesting an effect on primary, rather than secondary, memory. Finally, we have found that the scopolamine-induced impairment in recognition memory can be antagonized fully by physostigmine and partially by arginine vasopressin.

Based on previous results indicating that THC may be exerting its effects through an action on the limbic system, we administered this drug to monkeys performing an interval-timing task known to be sensitive to hippocampal damage. Doses of THC that we previously found to impair visual recognition memory produced shifts in the monkeys interval-timing abilities that were in the same direction as those observed following hippocampal damage.

In a series of experiments on habit formation, we administered the dopaminergic-neurotoxin MPTP to monkeys, producing learning and motor impairments that resolved within a few weeks. In spite of the recovery, the animals were more sensitive to the disruptive effects of scopolamine, suggesting residual damage to the dopaminergic system. Two years after the last dose of MPTP was given, substantial dopamine deficits were confirmed in these animals by positron emission tomography and biochemical analysis. At this time, the animals were impaired in learning a new task, detour reaching.

PROJECT DESCRIPTION:

The cholinergic system is now suspected to play a critical role in mnemonic processes both in humans and in primates. During the past year we have continued to study and identify the processes and functions in which acetylcholine influences memory in primates. Our earlier work showed that scopolamine, a muscarinic-cholinergic receptor blocker, impairs visual recognition memory in macaques performing a delayed nonmatching-to-sample task with trial-unique objects. We also showed that impairments were greater when scopolamine was administered before, rather than after, the acquisition trials, suggesting that cholinergic blockade mainly affects anterograde processes, influencing storage more than retrieval.

Experiment 1

We previously showed that recognition memory impairment could be produced in monkeys by combined but not by separate neurotoxin-induced lesions of the three major nuclei of the basal forebrain cholinergic system. In addition, these animals showed an altered sensitivity to cholinergic drugs compared to normal animals. The cells of the basal forebrain cholinergic nuclei, particularly those constituting the nucleus basalis of Meynert, the major source of cholinergic innervation of the cortex, form an extremely complex shape that renders them very difficult to locate and damage by standard stereotaxic methods. In our original studies, we used electrophysiological recording to identify the boundaries of the anterior commissure (AC), which then served as a reference for locating the basal forebrain nuclei. Although we obtained significant functional impairments with this method, the lesions were not complete and were highly variable. During the past year, we have continued our efforts to utilize magnetic resonance imaging (MRI) to help us visualize and locate the basal forebrain areas of interest. We designed and had built a nonmagnetic stereotaxic instrument for use in MRI and for neurosurgery. The initial scans showed even more detail than anticipated, largely because of the steadiness provided by the stereotaxic instrument. As a consequence, we have now been extremely successful at producing lesions of the basal forebrain nuclei based on the coordinates derived from the MRI. This method appears to provide a level of accuracy and reliability surpassing that obtained with other techniques, and promises to be applicable to many other problems as well.

Experiment 2

We have continued to use the automated testing apparatus that we developed in recent years and which is allowing us to study drug effects on memory in monkeys more rigorously than previously possible. We have begun to study the role of endogenous neuropeptides in memory processes as well. Arginine vasopressin (AVP) is one such peptide that has been reported to show reduced activity in Alzheimer's disease. Although facilitation of some retention processes in rodents and humans has been reported, there have been no systematic studies of AVP's effects on memory in monkeys. We first examined

the effects of AVP, administered alone, on a recognition task in the automated testing apparatus. Subcutaneous doses of AVP (1.0, 1.78, 3.2, and 5.6 ug/kg) produced an inverted U-shaped dose-effect curve, with 3.2 ug/kg producing a small, but nonsignificant improvement in performance. When AVP was administered prior to scopolamine, however, the peptide significantly attenuated the scopolamine-induced impairment, even though the scores did not return to control levels.

Experiment 3

We have now shown that scopolamine impairs performance of monkeys on a variety of object-memory tasks including recognition, recency, and recall. The results have indicated further that scopolamine affects primary memory, possibly acting to prevent information from entering even into an immediate store. We next determined if these findings applied to spatial memory, as well, by administering scopolamine to monkeys trained in delayed response with delay intervals ranging from 0 to 10 seconds. During the delays, an opaque screen was lowered to block the animal's view of the test tray, except on half of the 0-second-delay trials when the screen was not lowered. Although much higher doses (10, 17.8, 32, and 56 ug/kg) were required than in the object-memory tasks, scopolamine again produced a dose-related impairment in performance at all delay intervals except the 0-second delay without the screen, where it was ineffective. These results support our previous conclusion that scopolamine impairs the very earliest stages of information storage.

Experiment 4

Using in vitro autoradiography, we had previously mapped the distribution of both nicotinic and muscarinic cholinergic receptors in the cerebral cortex. Now, using a new antibody against choline acetyltransferase (ChAT), the enzyme responsible for the synthesis of acetylcholine, and a new perfusion technique, we have been able for the first time to visualize cholinergic fibers in the cerebral cortex. In addition, using a new computer system that allows us to superimpose sections labeled with [³H] nicotine, [³H] QNB, or ChAT over sections stained for cell bodies, we have been able to more accurately describe the laminar distributions of these receptor types and to compare them with the distribution of ChAT positive fibers.

To date, we have only analyzed one area, premotor cortex, in detail for all three markers. In this field, there is a dense band of muscarinic receptors in layers I-upper IIIa, and a second, less dense band in layers V-VI. Layer IIb was only slightly above background. Nicotinic receptors were seen only in the lower part of layer IIIa. ChAT positive fibers were seen in all cortical layers but were densest in layer II and the upper part of layer V. They were least dense in layer IIb. Fibers stained for acetylcholinesterase were similarly distributed.

The distributions of muscarinic and nicotinic receptors were also compared in other areas. In general, muscarinic receptors were most dense in the supragranular layers, although there was often a second less dense band in the infragranular layers as well. In the granular prefrontal areas, the distribution of muscarinic receptors was almost homogeneous across all the cortical layers. These may be the only fields in which muscarinic receptors are found in layer IV.

Nicotinic receptors, by contrast, are typically found in a single band in the middle layers of the cortex. In the primary somatosensory and auditory areas, for example, this band is found in the lower part of layer III and in layer IV, the layers of termination of the thalamocortical and feedforward corticocortical afferents to a given field. Only primary motor cortex had a different pattern. In this field, nicotinic receptors were distributed in a pattern similar to the one that was typical for muscarinic receptors. There was a dense band of receptors in the supragranular layers and a second less band in the infragranular layers with little, if any, label in layer IIb.

Nicotonic and muscarinic receptors thus have contrasting though not entirely complementary distributions in cortex. The nicotinic receptors are generally located in the layers of densest afferent input. The muscarinic receptors are typically seen above and below these layers. In the one area in which it has been examined so far, the distribution of cholinergic fibers more closely matches the distribution of muscarinic receptors, though this match is not exact and there clearly are fibers in the layer where the nicotinic receptors are located. The role that acetylcholine plays in modulating cortical function has yet to be determined. Descriptions of the normal distributions of cholinergic fibers and receptors are, however, important steps in understanding this modulatory function as well the perturbations that might occur following different types of insults to, or interference with, this system.

Experiment 5

We have continued our collaboration with the National Institute of Drug Abuse on the cognitive effects of delta-9-tetrahydrocannabinol (THC), the active ingredient of marijuana. We have reported previously that THC impaired recognition memory but not habit formation, suggesting a possible selective action of the drug on limbic structures. Indeed, a number of reports by others suggested that THC may have a deleterious effect on the hippocampus specifically. To examine this possibility further, we next studied the effects of orally administered THC on peak-interval timing, a test of temporal memory that has been shown to be especially sensitive to hippocampal lesions in rats.

Monkeys were trained in a computer-controlled procedure to press a lever in the presence of a white light for food reward. In each daily session, 75% of the trials were control trials in which the light was turned on and food was delivered immediately following the first response that occurred after an interval of 20 seconds had elapsed. The remaining trials were peak-interval

trials, which were identical to the control trials except that food was not delivered and the trial continued for a total of 50 seconds. After training on this procedure, the monkeys response rate increases to a maximum at the expected time of reward and then decreases in a fairly symmetrical fashion. Oral doses of THC (1, 2, and 4 mg/kg), which were shown previously to impair recognition memory, were administered 2 hours before the session. THC produced a dose-related peak-interval shift to the left, i.e., the remembered time of reinforcement was decreased. These shifts are in the same direction as those observed after lesions of the hippocampus in rats and suggest that the peak-interval procedure may provide a sensitive method for studying the actions of THC on this structure.

Experiment 6

We have also continued our study of the contributions of the neostriatum to habit formation. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), a neurotoxin that selectively destroys the nigrostriatal dopamine system when given in high doses, has been shown to impair motor function permanently in much the same way as that observed in Parkinson's disease. We had previously administered this drug to four monkeys that were tested both for motor function as well as concurrent discrimination learning with 24-hour intertrial intervals, a measure of the ability to acquire habits. The purpose was to determine whether there might be effects of the neurotoxin on learning unaccompanied by motor deficits. One pair of monkeys had been given MPTP in a series of small doses at approximately one month intervals. Discrimination learning was slowed after each dose increment, but deficits were most evident when the cumulative dose exceeded 3.8 mg/kg. Motor impairment was observed when the dose was increased to 4.3 mg/kg. Both learning and motor impairments resolved within two weeks after the last dose of MPTP. In the other pair of monkeys, MPTP was administered in higher doses and at shorter intervals (0.5 mg/kg, once per week for three weeks). Both of these monkeys developed severe movement disorders that persisted for two to three weeks before gradually resolving. Only one of these monkeys showed evidence of a learning impairment after this dosing regimen, although, when a fourth dose of MPTP was administered one month later, neither animal showed a learning deficit. These results suggest that the dopaminergic system had been damaged, but perhaps not sufficiently to prevent either recovery or compensation by remaining neurons. Since the dopaminergic system is known to compensate for neuronal loss up to a point, these animals may be more sensitive to challenge by other drugs or may even start to show deficits as they age, since dopaminergic activity is known to decrease with advancing age. To test these possibilities, we tested the same monkeys on another learning task, detour reaching. Even though the last MPTP injection had been given 18 months earlier and the animals appeared behaviorally normal, their performance on this simple task was significantly impaired. Positron emission tomography (PET) scanning revealed a substantial reduction from normal in 6-F-DOPA accumulation in the basal ganglia, indicative of terminal degeneration there in the treated animals. In

addition, CSF levels of dopamine metabolites were significantly reduced in these monkeys, and they were found to be extremely sensitive to administration of the neuroleptic haloperidol, tolerating only five days of treatment before developing severe extrapyramidal symptoms (akinesia and rigidity). Taken together, these results demonstrate that MPTP treatment severely damaged the dopaminergic system in these monkeys and suggest that further analysis of their learning abilities and their responses to drug challenge is clearly warranted.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Our results continue to provide convincing evidence that cholinergic mechanisms are critical for cognitive memory in monkeys. These mechanisms appear to play a more important role in storage than in retrieval and, further, the effect on storage occurs at a very early stage of processing. The finding that AVP attenuates the memory impairment produced by scopolamine has important implications for the pharmacotherapy of Alzheimer's disease. The finding that MPTP produces long-lasting impairment in dopaminergic function in spite of behavioral recovery has important implications for understanding the neurochemistry and plasticity of this system. Finally, our findings that administration of THC impairs timing ability in monkeys may provide the means to study the effects of this drug on hippocampal function.

PROPOSED COURSE OF RESEARCH:

We will continue to evaluate the effects of cholinergic and noncholinergic compounds, neuropeptides, and THC on cognitive memory and habit formation in monkeys. We will also begin to use the technique of in vivo microdialysis to study acetylcholine and dopamine function in behaving monkeys. In addition, we will continue to examine the roles of the basal forebrain cholinergic and nigrostriatal dopaminergic systems in cognitive and noncognitive memory, respectively, following damage to these two systems and to examine the possible compensatory actions of drugs following such damage.

PUBLICATIONS:

Aigner, T.G. Delta-9-tetrahydrocannabinol impairs recognition memory but not discrimination learning in rhesus monkeys. Psychopharmacol., in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00471-33 LPP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Heredity and Environment in Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Allan F. Mirsky, Ph.D.

Chief

LPP, NIMH

Co-PI: Loring J. Ingraham, Ph.D.

Senior Staff Fellow

LPP, NIMH

COOPERATING UNITS (if any)

Oranim Institute for Research on Kibbutz Education, Haifa University, Israel;
Hebrew University, Israel; University of Chicago, Illinois

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project has been composed of the following studies: (1) An intensive multi-disciplinary study of a family with MZ quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome; (2) Studies of Danish adoptees and their biological and adoptive families; (3) A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel. We maintain contact with the quadruplets but have not pursued active studies with them during the past two years. The Danish adoptees are of continuing interest to us and we are preparing additional reports on factors involved in their psychiatric outcome. The Israeli children are the subject of intensive research efforts and we are currently conducting further behavioral and biological studies with them.

DescriptionA. Other Personnel

Shaul C. Sohlberg, Ph.D.	Clinical Psychologist	Israel
Michael Natan	Psychologist	
Sol Kugelmass, Ph.D.	Oranim Institute	
	Professor of Psychology	
	Hebrew University	Israel
Joseph Marcus, M.D.	Professor of Child Psychiatry	Chicago,
	University of Chicago	Illinois
Eugene P. Tassone	Psychologist	LPP/NIMH
Olive W. Quinn, Ph.D.	Guest Researcher	LPP, NIMH
Patricia Lowing, Ph.D.	Private practice	Michigan
Deborah Levy, Ph.D.	Director Psychophysiology	New York
	Lab, SUNY Stonybrook	

B. Objectives

The project is composed of the following studies: (1) An intensive multi-disciplinary study of a family with MZ quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome. We are continuing our contacts with this family to see what happens in the clinical course of these women and to see how the course is related to earlier and to current life experiences; (2) Studies of adoptees and their biological and adoptive families in Denmark; (3) A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel.

C. Major Findings

The objectives of this project are to understand how hereditary and environmental factors interact to make for schizophrenic outcomes of varying types and degrees.

1. The Genain Quadruplets

Our recent studies of the Genain quadruplets are summarized in the annual reports of the previous five years. This year, there was one publication of a summary of prior studies of these women in a monograph on aging and schizophrenia. A second publication now in press will be included in a special issue of the Schizophrenia Bulletin dedicated to long-term followup studies in schizophrenia. In this review we discussed a number of matters, including the evidence linking the psychiatric outcome of the Genain quadruplets to the information regarding perinatal brain damage; available data (supported by the 1981 studies) suggests that Nora and Hester sustained the greatest injuries at birth. This edition of the bulletin was edited by Drs. William T. Carpenter and Thomas H. McGlashan. Dr. Olive Quinn, who is a Guest Researcher in the LPP, maintains contact with the Genains and is a coauthor of the two reviews referred to above. Interest in the quadruplets continues to be high, as judged by the steady demand for photographs of them for psychology text books.

Several years ago, Dr. Deborah Levy visited the Genains and made smooth pursuit eye movement recordings of all four women in their home. The results of these measurements have been incorporated in the Schizophrenia Bulletin article.

2. The Danish Adoptee Study--Reanalysis of the Data

Using data from Danish health records, in a now-classic study, Rosenthal, Kety and Wender compared the frequency of schizophrenia spectrum disorders in two groups of persons adopted in infancy or early childhood: those with a psychotic parent (index group) and those whose biological parents had never had psychiatric treatment (control group). Significantly more disorder was found in the index than the control group. We have recently completed a reanalysis of the extensive interview data collected on this group of subjects, focusing on stressful events during childhood and adult outcome. In a report we have submitted for publication, we present evidence that more stressful events were found in the childhood histories of index subjects who developed schizophrenia or schizotypal personality disorder (SPD) than in the histories of index subjects free from these disorders, and that greater number of stressors are associated with more severe outcomes. In matched control adoptees, of biological parents free from psychiatric illness, we found less psychiatric illness but an equal number of stressful events during childhood when compared to index adoptees. These results are consistent with a stress-diathesis model, where both genotype and environment contribute to adult illness.

Further analyses of these data revealed that the most common stressors among index subjects with schizophrenia or SPD were troubled family environments. These results are consistent with the current body of literature investigating expressed emotion as a factor in the relapse of adult schizophrenic patients.

3. The Israel Kibbutz--High Risk Study

In 1984, the Laboratory published in the Schizophrenia Bulletin a report of work begun in 1962 on the study of children at risk for schizophrenia in Israel, which was designed and initiated by David Rosenthal. The study has examined 100 children, of whom 50 had one schizophrenic parent (index subjects), and 50 were born to two nonschizophrenic parents (control subjects). Half of both the index and control groups were reared in towns in traditional nuclear families, while the remaining half were reared in communal settings on kibbutzim.

In broad outline, the results indicate that index children were discriminable from controls in many areas of function, but kibbutz and town children did not differ on the experimental examinations. Furthermore, kibbutz versus town rearing had no discernible effect on the performance or behavior of the high-risk index children. Index children were found to be poorer in psychosocial adjustment, perform more poorly in school, manifest a

number of neurological "soft signs," and show deficits on psychological tests requiring high levels of attention, visual integration, and visuomotor coordination. An important negative finding was lack of differences between index and control children on psychophysiological measures of arousal and habituation in the first examination.

We have also conducted (in 1981) followup interviews with the study subjects whom were then in their mid-twenties, at the peak of their risk period for schizophrenic breakdown. Ninety of the surviving 99 subjects were seen. Results showed that nine subjects fell within the "schizophrenia spectrum" (of whom six had DSM-III Schizophrenia), six from kibbutz backgrounds, and three from towns. When all DSM disorders were considered, more than five times as many ill subjects fell within the index (N=23) than within the control group (N=4). Furthermore, when schizophrenia itself was excluded, the remaining subjects with history of illness (including DSM-III Major Affective Disorder or Dysthymic Disorder) were found predominantly in the index-kibbutz cell (16 of the total of 23 in the cell, including 9 with affective disorder). Other significant results included persistence of attention-related deficits in the index group, and continued poor social and work adjustment in high-risk subjects.

During 1987-1988 we have initiated, and nearly completed, a second psychiatric diagnostic followup of these subjects, who are now in their early thirties. At this time, the majority of the subjects who will develop a schizophrenic disorder should have become ill. In order to diagnose these subjects accurately, a Hebrew version of the SADS-L, with modifications allowing the accurate assessment of schizophrenia spectrum disorders, has been developed. Social workers who are blind to the subjects' index or control status have been trained in its use. In addition, our laboratory has developed and validated Hebrew versions of neuropsychological tests (including the Stroop test) for use with this population. One of these Hebrew-version tests has been published. Subjects have been contacted and are currently being interviewed. As of this writing, 77 of the original subject cohort have been seen as well as approximately 30 of the parents. The renewed contact with the parents is important to reaffirm that the original (circa 1964) diagnoses were substantially correct. With respect to the probands themselves, our data so far do not indicate that more schizophrenic illness has developed between 1981 and 1988; on the other hand, more subjects with affective disorders have been identified and these subjects have been found in both index and control groups.

Significance to Biomedical Research and to the Program of the Institute

The issue of the mode of heritability of schizophrenia, and factors which modify its development, may be the highest priority of the Institute. This work contributes significantly to our knowledge in this area and ultimately, to our capacity to treat and prevent schizophrenia and related disorders. Our study of childhood stress and adult schizophrenia spectrum disorder suggests a possible direction for prevention of adult psychiatric morbidity.

The studies here, which focus on schizophrenia spectrum disorders as well as pure DSM-III Schizophrenia, aid in the identification of milder syndromes genetically linked to schizophrenia. The identification of such syndromes would aid in the search for clear pedigrees of schizophrenic illness by allowing more individuals to be studied and tested for biological markers than the current low number of biological relatives with frank schizophrenia.

Proposed Course

Genains: Drs. Quinn and Mirsky maintain contact with the Genains and exchange correspondence on an occasional basis. We are considering the possibility of inviting the Genains back to NIMH for another series of followup studies to include electrophysiological and other measurements that were not obtained during their visit in 1981.

Denmark: We have begun work on a followup study of the Danish adopted away offspring of schizophrenic parents first studied by Rosenthal. The group of Danish investigators is supportive and enthusiastic about this, and have begun to search for current information about the adult outcome of these adoptees. At the time of their previous assessment, a portion of the subjects was too young to have passed through the major risk period for schizophrenia. This study will allow for continuing investigation of the longitudinal course of schizophrenic illness as well as evidence on the role of environmental factors, including stressful events, on the development and course of schizophrenia.

Israel: In addition to the current followup study of this population, our laboratory has also taken steps to validate previously reported findings from this unique sample. We are planning to interview the siblings of the probands in this study in order to increase economically the sample size and the power of statistical tests conducted with this sample. In addition, we have begun to reinterview the parents in order to use contemporary diagnostic tools to confirm the earlier diagnosis of schizophrenia in index subjects' parents. In addition, family history interviews of these families, now being obtained, will help clarify the role of familial schizophrenic and affective illness in the development of psychopathology among these subjects.

As the interviews are completed, we will be collecting neuropsychological test data as well as CT images from consenting subjects. This information will also build on earlier studies of this population to present a fuller picture of the psychobiological and environmental factors leading towards adult psychiatric illness.

Expressed emotion: Our current findings in the Danish sample, taken in conjunction with the developing literature on expressed emotion, suggest that further exploration of the social environment of adult schizophrenic patients may be useful in identifying modifiable environmental factors that could reduce the incidence of relapse. We are currently developing an approach to the study of Expressed Emotion that would shift the focus away from the parents of schizophrenic adults living at home and place it on the social

environment of the larger number of schizophrenic patients who live outside of their family of origin. We believe that this approach will lessen the potential for inappropriate stigmatizing of the families of schizophrenic patients, and lead to more effective support strategies for adult schizophrenic patients.

Publications

Mirsky AF, Quinn OW, DeLisi L, Schwerdt P, Buchsbaum M. The Genain quadruplets: a 25 year follow-up of four monozygous women discordant for the severity of schizophrenic illness. In: Miller ME, Cohen GD, eds. Schizophrenia and Aging, New York: Guilford Publications, 1987;83-95.

Mirsky AF, Quinn OW. The Genain quadruplets. Schizophr Bull 1988, in press.

Marcus J, Hans SL, Nagler S, Auerbach JG, Mirsky AF, Aubrey A. A review of the NIMH Israeli kibbutz-city study and the Jerusalem infant development study. Schizophr Bull 1987;13:425-38.

Mirsky AF. The Israeli high-risk study. In: Dunner DL, Gershon ES, eds. Relatives at Risk for Mental Disorders. New York: Raven Press, 1988; 279-98.

Mirsky AF. Research on schizophrenia in the laboratory of psychology and psychopathology, 1954-1987. Schizophr Bull, 1988, in press.

Ingraham LJ, Chard F, Wood M, Mirsky AF. A Hebrew language version of the Stroop test. Percept Mot Skills 1988, in press.

Myslobodsky MS, Ingraham LJ, Weinberger DR. Skull asymmetry and handedness in adults: a possibility of their association with lateral head turning in infancy. Percept Mot Skills 1987;65:415-21.

Ingraham LJ. The modes and morals of psychotherapy, 2nd ed. In: London P (book review). Sci Book and Film 1987;22:148-9.

Ingraham LJ. Stature and stigma. In: Martel LF, Biller, HB (book review). Readings 1988, in press.

Ingraham LJ. Psychotherapy of schizophrenia. In: Benedetti, G (book review). Sci Book and Film 1988;23:285.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00484-28 LPP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychophysiological Responsivity and Behavior in Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Theodore P. Zahn, Ph.D. Research Psychologist LPP, NIMH

COOPERATING UNITS (if any)

Laboratory of Socio-Environmental Studies, Child Psychiatry Branch, Laboratory of Clinical Science, Neuroscience Branch, Biological Psychiatry Branch, and Clinical Neurogenetics Branch, NIMH.

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.3

PROFESSIONAL:

0.9

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The general purpose of this project is to investigate the roles of autonomic nervous system (ANS) activity, attention, and information processing and their interrelationships in the pathology, etiology, and prognosis of psychiatric disorders. A second purpose is to determine biological and psychological processes related to ANS activity and attention. ANS activity is assessed by peripheral measures, such as skin conductance, heart rate, and skin temperature. Subjects are tested under conditions of rest, presentation of tones, and performance on tasks such as reaction time and mental arithmetic.

Biological mechanisms are investigated by correlating these variables with enzyme activity, neuropeptides, and levels of biogenic amines and their metabolites and with brain dysfunction as revealed by CT and PET scans.

Studies are being done on unmedicated patients with diagnoses of schizophrenia, affective disorder, obsessive compulsive disorder, anxiety-panic disorder, and autism to test the diagnostic specificity of patterns of ANS activity. Children of parents with bipolar affective disorder are being studied to determine possible ANS trait markers. In some studies blood samples are taken during ANS recording sessions in which stressful procedures are given. In one, the effects of success and failure to escape an aversive noise are assessed, and in another, the effects of a dose of yohimbine is being studied. Clinical trials of various treatments are studied in various groups.

Psychological correlates are studied via clinical background data, clinical ratings and questionnaires, and by procedural variations, and by correlations with tests of attention, information processing, and cognition.

Project DescriptionA. Other Personnel

Allan F. Mirsky, Ph.D.	Chief	LPP, NIMH
Carmi Schooler, Ph.D.	Acting Chief	LSSES, NIMH
Dennis Murphy, M.D.	Chief	LCS, NIMH
David Pickar, M.D.	Chief	SCS, NSB, NIMH
Thomas Uhde, M.D.	Staff Psychiatrist	BPB, NIMH
Judith Rumsey, Ph.D.	Staff Fellow	CHP, NIMH
Judith Rapoport, M.D.	Chief	CHP, NIMH
Frank Putnam, M.D.	Staff Psychiatrist	LDP, NIMH
Wade Berrettini, M.D.	Senior Surgeon	CNG, NIMH
Christine Ollo, Ph.D.	Staff Fellow	BPB, NIMH
David Rubinow, M.D.	Clinical Director	BPB, NIMH

B. Objectives

The major objective of this project is the further understanding of the role of autonomic nervous system (ANS) activity, information processing and attention, and their interrelationships in psychiatric disorders, primarily schizophrenia. The overall strategy involves studies of ANS and attentional relationships to diagnosis and prognosis, studies of the effects of drugs and other therapeutic interventions, "high risk" and personality studies in normal volunteers, studies of the effects of various types of stress, and studies of the measurement of ANS activity.

C. Methods Employed

The general methods of these studies include measurement of ANS activity through skin conductance (SC) usually measured bilaterally, heart rate (HR), vascular activity (skin temperature and finger pulse volume), and respiration while subjects are resting, exposed to a series of nonsignal tones of constant or variable intensity, and performing tasks. Tasks include tests of attention using reaction time techniques, tests of perceptual speed, and tasks designed to be moderately stressful. A mini-computer system is used to run the experiments and to collect and analyze the data. Studies in various stages of completion are listed below.

1. Schizophrenia Studies

a. A study of newly admitted, drug-free patients used three tasks varying in stressfulness and task demands to test the hypothesis, developed in previous studies, that schizophrenics' ANS does not respond appropriately to variations in stimulus significance. This study also includes several rest periods and a series of nonsignal tones for comparative purposes.

b. In current studies, ANS recording is being carried out in two sessions which include rest periods, a tone series, and two reaction time tasks. In addition, several methods of assessing more precisely the nature of

schizophrenic attention deficits using reaction time (RT) techniques are being compared. (See Z01 MH 00484-25 LPP, 1984-85 for details.) Patients are being tested on standard neuroleptic medication as well as drug free. The purpose of this is to compare our results to most of the recent published non-NIH studies which, for the most part, use medicated patients. Some patients have been tested in the "learned helplessness" paradigm described in section 3b below.

c. Tests of the ANS effects of drugs such as pimozide, lithium, naloxone, GHB, verapamil, propranolol, and prazosin, and treatments such as hemodialysis and plasmapheresis, have been carried out.

2. Studies on Nonschizophrenic Psychopathology

a. Several confirmed psychophysiological "markers" of schizophrenic pathology have been detailed in previous annual reports and are summarized below. In order to determine which of these are specific to schizophrenia, patients with other types of psychopathology are being tested on the initial part of the current standard protocol (described in 1.b. above) after being medication free for an appropriate time. These include patients with major depressive, obsessive-compulsive, and panic-anxiety disorders (see Z01 MH 00071 BP, 02184 NS, 00153 CHP, and 00336 LCS). In addition, a group of young men who had a diagnosis of early infantile autism have been studied (see Z01 MH 00178 CHP). Some patients with affective disorder and premenstrual syndrome have been tested in the "learned helplessness" paradigm described in section 3b below.

b. Drug effects are being evaluated in several groups. Some panic-anxiety patients are tested on imipramine (double-blind) and will be compared with patients given placebo, and some we are able to test under both treatments. We have completed a study of the psychophysiological and attentional effects of a challenge with yohimbine--a noradrenergic alpha-2 antagonist on patients with panic disorder and normal controls in collaboration with Drs. Albus of NSB (now returned to Munich, F.R.G.) and Uhde of BPP.

We have nearly completed data collection on a double blind crossover study of the effects of clomipramine and desipramine in OCD children and have started an assessment of the effects of long term clomipramine treatment of this group. Clomipramine has been found to be highly effective, and other tricyclics ineffective treatments in OCD. In a previous study on adults with OCD we obtained different psychophysiological effects for clomipramine and clogyline, an ineffective treatment. Our current study should be a partial replication and extension of this.

c. Some studies are assessing state changes independently of pharmacological treatment. Earlier annual reports described studies of state changes in multiple personality patients. In collaboration with CHP we have done follow-up studies on formerly adolescent patients with obsessive-compulsive disorder. These patients should have different levels of current

symptoms, allowing us to separate out "state vs. trait" influences. Similarly, a small group of euthymic bipolar affectively disordered patients have been studied in the "learned helplessness" paradigm and compared with symptomatic patients.

d. As part of a larger LPP project, we are testing subjects with known brain lesions from head injuries on the standard ANS and attention protocol used with schizophrenics. The purpose is to determine what specific brain areas may be involved in schizophrenic psychopathology.

e. A study on the psychophysiology and neuropsychology of the premenstrual syndrome (PMS) in collaboration with Drs. Ollo and Rubinow of BPB is in the planning stage. Tentatively we plan to study psychophysiological activity in women with a diagnosis of PMS in both follicular and luteal phases in a protocol involving relaxation and stress periods. An attempt to exacerbate the effects of stress by playing personal tape recordings of descriptions of distressing PMS symptoms is being planned.

3. Studies on Normals

a. In collaboration with CNG, we have tested the offspring of patients with bipolar affective illness to determine if some of the putative ANS trait markers for affective disorder reported in the literature can be considered to be genetic markers.

b. A collaborative study with NSB is designed to compare temporary states of "learned helplessness" and active coping on learning, mood, ANS activity, plasma catecholamines, and cortisol. Subjects are presented with 60 aversive (100db) tones (maximum 5 sec) and given the task of learning to turn them off. In one condition (nonscape), the problem is insoluble and in the other (escape), it can be solved by correctly pushing a button. Subjects receive the same instructions for both conditions and the total amount of noise is equated by means of a "yoking" procedure. The object is to study the neurobiology of a temporary model depressed state in humans.

4. Major Findings

a. Schizophrenic Studies

1. Confirmation of previous findings of high autonomic base levels and a sluggish response to the mild stress of task performance in schizophrenics was observed and was more extreme in eight patients with significant cortical atrophy as shown by CT scan. These and other findings have been detailed in previous annual reports.

b. Data are still being collected. The new data continue to show that the phenomena of visual sensory dominance and intersensory facilitation found in normal subjects also occur in schizophrenics. A subsample of these patients have been used as a comparison group for the autism study described below.

2. Studies on Nonschizophrenic Psychopathology

a. Results for obsessives and autistics were presented in some detail in previous annual reports have been published. Both groups showed distinct patterns of ANS activity across the various conditions which differed from their respective control groups and from schizophrenics. Data for other groups are still being analyzed.

Preliminary results of the following study of obsessive adolescents were detailed in last year's annual report. They show, paradoxically, stronger relationships between current clinical state and the original physiological variables of speed of habituation and baseline heart rate then for current state and current psychophysiology. More detailed analyses of these data are planned.

b. Results for the yohimbine challenge were described more fully in previous annual reports. Briefly, yohimbine produced differential effects compared to placebo on mood and heart rate in patients with panic disorder than in controls irrespective of whether the patients were being treated with alprazolam. Heart rate variability and serum cortisol increased about the same amount in both groups and SC activity was not greatly affected by the drug in either group. The heart rate variability data suggest that hyperventilation may contribute to the genesis of panic attacks.

Recently the plasma norepinephrine (NE) data became available. All groups showed about the same significant increase in plasma NE after the yohimbine challenge compared to placebo regardless of diagnosis or treatment. The greater effects on heart rate might suggest greater beta receptor sensitivity in patients with panic disorder. However, yohimbine's effects on NE and on heart rate were uncorrelated in the patients (unlike the positive correlation found in controls), so the heart rate effects could also be secondary to the greater subjective effects of yohimbine in the patients.

c. The multiple personality project results were reported previously and will be prepared for publication.

d. In preliminary analyses, the Alzheimers patients showed generally a strong trend for hyporesponsivity in skin conductance but because of one or two outliers in each group, these results are not significant.

3. Studies on Normals

a. Our completed analyses on data from 22 subjects at genetic risk for affective disorder (Risk group) and 27 controls, 15 to 25 years old have confirmed and strengthened with adequate statistical treatment, the preliminary results detailed in last year's annual report. The working hypothesis which stimulated this study namely, that low electrodermal activity (EDA) is a trait marker for affective disorders, was definitely not confirmed. Nonsignificant differences in the opposite direction were observed at rest, and during the

most stressful parts of the protocol, including a mental arithmetic task, one index of EDA was significantly higher in the high risk groups. Another finding was that during task periods (reaction time and mental arithmetic) EDA was significantly lateralized to the left hand in the high risk group compared to controls. This is of interest because of similar reports in the literature on symptomatic patients. Lastly, the high risk subjects did not differ from controls on self ratings of anxiety or anger during the protocol, but did show significantly greater ratings of depression during the mental arithmetic procedure. Although this study has disconfirmed the hypothesis that low EDA is a genetic marker for affective illness, it suggests EDA hyperactivity to mild stress, atypically lateralized EDA, and a tendency to become depressed under mild stress as potential markers for risk for affective illness. Planned followup evaluations of this cohort will enable us to test the actual predictive utility of these markers. This study was presented at two meetings last year and a paper is nearly ready for submission.

b. Results for the first 10 control subjects in the learned helplessness study were reported previously. The major findings were greater elevations of ACTH, electrodermal activity and dysphoric mood following the nonescape procedure. A paper on these subjects has been published.

We have done some preliminary analyses on the psychophysiological data for a larger group (N=19) of controls and have compared them with 14 unmedicated patients with affective disorders. Subjects had significantly higher background autonomic activity in terms of spontaneous electrodermal fluctuations, skin conductance level (SCL) and heart rate during the nonescape compared to the escape condition, confirming the more stressful nature of the lack of ability to control the noise. The difference in SCL persisted to a subsequent period during which another task was given. There were no differential effects of diagnosis on these findings, suggesting that patients with affective disorder respond about the same way to these procedures as do controls in terms of ANS activity. Patients who were symptomatic had significantly less EDA than controls and euthymic patients independently of the situation, suggesting that low EDA may be state-related. A small group of euthymic bipolar patients (N = 5) gave evidence of EDA hyperresponsivity to both escape and nonescape procedures. If this turns out to be significant it will confirm the finding high risk study that ANS hyperresponsivity is a trait marker for affective illness. Another tentative implication of these data is that the "learned helplessness" procedure produces a pattern of ANS activity that is clearly distinguishable from that occurring in patients with symptomatic affective disorder. We plan to present some of these data at a meeting in the fall.

Significance to Biomedical Research and the Program of the Institute

Investigations of ANS activity and attention in psychiatric disorders, especially schizophrenia, have produced promising results which suggest that these processes may play fundamental roles in the etiology and expression of the disorders. Limitations on inferences to be drawn from measures of ANS activity come from incomplete understanding of their biological and

psychological determinants. One of the main goals of this research is to increase this understanding by investigations of biological and psychological correlates and improving measurement techniques. The dynamic nature of these measures permits the study of processes such as adaptation, habituation, response to and recovery from stress, and effects of single stimuli through noninvasive techniques. Thus, further understanding of their mechanisms could greatly increase their utility in investigations of psychopathology. Continued investigations of the diagnostic specificity of these processes and of their relationships to other clinical features and to prognosis are necessary to confirm and extend our previous results and to test the limits of their generality.

Proposed Course

Analysis of data will continue for the completed project on schizophrenia with the goals of determining the relationship of ANS variables to diagnosis, task performance, and a number of clinical and biological variables available on these patients. Concept modeling by confirmatory factor analysis may be extended to this group.

Collection and analysis of data will continue for current projects on schizophrenic and nonschizophrenic psychopathology and in normal controls. We hope to have sufficient data this year to make a formal test of the diagnostic specificity of ANS activity. Much analysis remains to be done on the data from the "learned helplessness" study.

We are still hoping to test children in the Hopkins preventive intervention project whom LPP is negotiating to bring in on the same test protocol as we used previously with hyperactive, obsessive, and anxious children, and currently with children having a diagnosis of conduct disorder (see Z01 MH 00486 LPP).

We are planning to design a new protocol for schizophrenics with which to test the hypothesis that schizophrenics and controls exhibit differential effects of increases in arousal on attention. Arousal will be manipulated by changes in posture and variations in stimulus intensity and significance.

We are putting together an eye tracking system with which we plan to study the role of attentional factors in saccadic reaction time and accuracy and smooth pursuit tracking in various patient groups and normal controls.

Publications

Zahn TP. Studies of autonomic psychophysiology and attention in schizophrenia. *Schizophr Bull*, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00486-16 LPP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychophysiological Effects of Stimulant Drugs in Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Theodore P. Zahn, Ph.D. Research Psychologist LPP, DIRP, NIMH

Other: Judith Rapoport, M.D. Chief CHP, NIMH

Marcus Kruesi, M.D. Clinical Associate CHP, NIMH

COOPERATING UNITS (if any)

Child Psychiatry Branch, NIMH

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH/ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Tests of the effects of acute and chronic administration of caffeine on autonomic nervous system (ANS) functioning have been carried out to evaluate the role of ANS activity in the behavioral and subjective effects of this drug. A test of attention using a reaction time method is included.

The test protocol involves recording peripheral indicators of ANS activity such as skin conductance (SC), heart rate (HR), and skin temperature during a session consisting of a rest period, presentation of a series of simple tones to which no response is required, and the reaction time task. Studies have been carried out on the effects of the acute administration of two doses of caffeine and a placebo in 6-13 year old boys and in men, and a study of chronic (2 week) caffeine intake in children.

The effects of both acute and chronic administration of caffeine were increases in SC indices of arousal but some trends toward decreases in HR. The SC results are consistent with the hypothesis that caffeine can be considered a pharmacologic model for anxiety, but the HR effects suggest the model is imperfect.

The most recent study, an acute dose protocol with caffeine was conducted on children with anxiety disorders and controls. This tested the hypothesis, for which there is evidence in adults, that patients with anxiety disorders are more sensitive to caffeine than controls.

Another current study compares ANS activity and attention in boys with diagnoses of Conduct Disorder and Attention Deficit Disorder.

Project Description

This project has evolved from the study of hyperactivity in children (now called Attention Deficit Disorder) to the study of stimulant drugs--dextroamphetamine and caffeine--in children and adults.

Results of the caffeine studies with normal subjects have been presented in previous annual reports. The acute dosage studies were published last year. The pattern of ANS results for caffeine was different from that of another "stimulant drug"--dextroamphetamine--in that caffeine produced very consistent and strong increases on SC activity but minimal or opposite effects in HR, while amphetamine dramatically increased HR and had less consistent effects on SC activity (although it also generally increased it).

In the recent study of children with anxiety disorders, as in our previous studies, caffeine increased SC activity and decreased HR. Contrary to expectation, caffeine effects on SC activity were not greater in the anxious children. On the contrary, caffeine produced more electrodermal activation in the controls, significantly so in some cases. These effects could not be accounted for by a difference in placebo values as these were not generally much different. In contrast, the anxious subjects showed a somewhat greater decrease in heart rate than controls, but this might be due in part to higher heart rate on placebo in the anxiety group. Side effects were not different in the two groups (see Z01 MH 00161 CHP).

Testing of boys with diagnoses of conduct disorder (CD) and attention deficit disorder (ADD) is being done with two general objectives in mind. One is to look for ANS markers of diagnosis. Previous research in this laboratory and others has shown that ADD boys do not differ from normals in indices of arousal but have generally lower ANS responsivity to stimuli. The literature on psychopathic personality in adults--a possible outcome of CD--shows evidence of low SC levels and diminished SC (but not HR) reactivity to nonsignal stimuli. Our ANS protocol will allow tests of these differences in the boys. A second objective is to explicate the attention deficit in ADD children and to test whether CD boys have similar deficits. A battery of simple and choice reaction time tasks, similar to those given to schizophrenics are being used for this purpose (see Z01 MH 00484-25 LPP, 1984-85).

Significance to Biomedical Research and the Program of the Institute

The ANS effects of caffeine consistently found in these studies partially resemble those seen in anxiety states and other psychopathology. Since caffeine effects are thought to be mediated by blockade of adenosine receptors, these studies may help determine the mechanisms involved in those aspects of ANS activity that are components of anxiety states. Our finding that children with anxiety disorders are not especially sensitive to caffeine effects is surprising in terms of the usual models of caffeine effects in adults with anxiety disorders and suggests either that these models do not apply to children or that they generally need revision.

The study on CD and ADD boys may also help determine the mechanisms of ANS measures through correlation with the extensive neurobiological data being obtained in this group. It should also provide evidence of a possible biological basis for the diagnostic distinctions. This study is relevant to a major objective of LPP to develop a taxonomy of attention disorders.

Proposed Course

Continued collection of data on the CD-ADD project is planned. The analysis of the data will include correlation of the ANS and attention data with metabolites of biogenic amines from CSF.

Publications

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00491-12 LPP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

~~Personality Factors and Psychophysiological Responses to Changing Stimulus Input~~
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Theodore P. Zahn, Ph.D. Research Psychologist LPP, NIMH

Other: Thomas N. Robinson, Jr. Guest Researcher LPP, NIMH

COOPERATING UNITS (if any)

NIH Normal Volunteer Office.

LAB/BRANCH

Laboratory of Psychology and Psychopathology

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INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project are to investigate relationships among differences in personality, sensory thresholds, motor responding, and autonomic nervous system (ANS) activity in normal humans. Bilateral skin conductance and heart rate have been recorded in sessions in which constant and variable intensity tones and lights are presented, in which auditory and two-flash thresholds (TFT) determined by methods which permit signal detection analyses and in which simple reaction time is measured. Several standardized personality tests were also given. These include scales of sensation-seeking, extraversion, neuroticism, psychoticism, field dependence and anxiety. In addition comprehensive measures of lateral dominance have been given. A procedure for manipulating ANS arousal experimentally with minimal distracting effects--a change in posture from supine to standing--is being used to study such problems as the effects of base levels on responsivity, the effects of arousal on performance, and the effects of personality variables on this relationship. This project allows testing of several theoretical models of the relationships of ANS activity, sensory sensitivity, and personality, some of which have implications for the etiology of psychopathology. Tests of the relationships between laterality in skin conductance variables and behavioral laterality will also be done to see if inferences about lateralized brain function can be made from such variables.

Project Description

A. Objectives

A large body of psychological literature postulates that an important dimension of individual differences in behavior or personality is reflected in the reactions of the nervous system to sensory stimulation. Pavlov's original conception of "strong" and "weak" nervous types has been modified and extended by Western theorists to reflect such personality dimensions as "extraversion-introversion," "sensation-seeking," and "field dependence," each of which can be measured by a questionnaire or other test procedures. The theoretical models that have been built up from these concepts have implications for interrelationships among personality, autonomic nervous system (ANS) base levels and responsivity to stimulation, and sensory sensitivity. There are also implications for psychopathology, in that schizophrenics have been considered to be extremely "weak" nervous types in the Pavlovian system (i.e., overreactive to weak stimulation and underreactive to strong stimulation--"transmarginal inhibition"). Another development is the more recent delineation by H. Eysenck of the dimension of "psychoticism."

The major objective of this project is to test some of the implications of these models of personality by interrelating the personality measures with sensory thresholds and sensitivity, motor responding, and ANS activity in normal humans. Other objectives are to explore relationships of differences in the laterality of skin conductance activity with behavioral assessments of laterality, and to test the effects on ANS activity increasing arousal by means of a postural change.

B. Methods Employed

Over 200 normal volunteers have been assessed on several personality dimensions, including the three Eysenck scales of extraversion, neuroticism, and psychoticism in addition to, field dependence, sensation-seeking, impulsivity, ego strength, and anxiety, assessed for degree of lateral dominance, and given tests of ANS and sensory functioning in the various protocols described earlier.

More recently a fixed foreperiod reaction time procedure similar to that used with patients in Z01 MH 00484 LPP was used.

C. Major Findings

In previous annual reports, relationships between questionnaire-defined personality variables, ANS activity, and sensory thresholds have been described. In general, subjects with high scores on the Eysenck personality scales of extraversion, psychoticism, and, surprisingly, neuroticism tend to have low ANS activity and reactivity. Low ANS activity was also found in subjects with high scores on a scale of schizotypal personality. Subjects high on sensation-seeking were also very responsive autonomically to novel stimuli.

During the past year we have focussed on the relationship between personality variables and reaction time (RT) and associated psychophysiological variables in a study involving 20 normal volunteers tested in a reaction time protocol with fixed foreperiods of 4 and 8 sec. along with ANS recording. Results show that introverts had significantly slower RTs than extraverts, particularly at the longer foreperiod. Other results show that two measures of impulsivity were negatively correlated with RT, and that extraverts who were high in the Eysenck scale of Psychoticism were faster than extraverts low on that scale whereas psychoticism had no effects on the RTs of introverts. Since psychoticism and extraversion are both related to impulsivity this suggests particularly fast reaction times in a group of impulsive extraverts. It is consistent with our earlier findings from psychophysiological data that the psychoticism scale is more closely related to psychopathy than to schizophrenia, since schizophrenics have slow RTs.

Introverts had more electrodermal activity during the instructions and practice trials for the RT task and showed relatively less deceleration in heart rate during the long foreperiod. This latter measure has been associated with focussed attention. Thus the slow RTs of introverts might be attributed to greater distractibility and/or less focal attention, similar to speculations about schizophrenia.

These results from a rather small sample of healthy subjects seem promising from the standpoint of a model of the variables affecting RT in pathological states such as schizophrenia.

Significance to Biomedical Research and the Program of the Institute

The construct of the strength of the nervous system, whatever it may be thought to denote in terms of neuronal functioning, has a traditional relevance to psychopathology in that a weak nervous system is said to be characteristic of schizophrenia. The association with the Western measures of introversion and low ego strength is also relevant in this context, as are the associated phenomena of enhanced sensory sensitivity and elevated ANS activity. Although there are wide subtype differences among schizophrenics, the concept of a schizophrenic characterized by social withdrawal, weak ego boundaries, and high arousal who is overwhelmed by environmental stimuli has been associated with the early stages of the illness. The data from this project appear to support the existence of a similar syndrome in normal subjects, albeit to a lesser degree. Thus this approach may be considered as an alternative to the increasingly popular "high risk" study using scales designed to measure some form of schizotypy by assessing symptom-like phenomena more directly.

Increased understanding of the relationships among autonomic, perceptual, and personality variables in normal subjects should be of great assistance in interpreting the autonomic and perceptual results from studies on psychopathology in which similar methods are used. This project has been very useful in the development of protocols for studies of psychopathology.

Proposed Course

A priority is to attempt to delineate further the strength of the nervous system construct. To this end multivariate analyses such as multiple regression and/or confirmatory factor analysis will be applied to this large data set. We also plan to do more data collection and analysis with the newer reaction time protocol.

We have some as yet unanalyzed psychophysiological data collected in two "high risk" studies in which extremely poor or good performance on either the Continuous Performance Task or pendulum eye tracking (both of which are impaired in schizophrenia) were selection variables. These data are obviously quite relevant to the questions discussed here and we plan to analyze them.

We are planning also to develop a new battery of tests that vary in their sensitivity to arousal, making use of some of the recent developments in the field of cognitive psychology.

Publications

Robinson TN, Jr. and Zahn TP. Preparatory interval effects on the reaction time performance of introverts and extraverts. *Pers Indiv Diff*, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00503-08 LPP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Clinical Studies of Attention Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Allan F. Mirsky

Chief

LPP, NIMH

COOPERATING UNITS (if any)

Epilepsy Branch, NINCDS; Clinical Neurosciences Branch, NINCDS; Laboratory of Clinical Sciences, NIMH; Neuropsychiatry Branch, NIMH; Boston University

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.25

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This research comprises three related areas of investigation concerned with specifying neuropsychological factors underlying clinical conditions in humans in which disturbed attention is a major symptom. A major emphasis is on (1) illuminating the nature of brainstem pathophysiology, if any, in such entities as petit mal or absence epilepsy, infantile autism, schizophrenia, and related diseases; (2) an additional major emphasis is on extending the neurobehavioral analysis of attention loss in absence epilepsy so as to facilitate developing alternative treatment strategies for such patients. Both of these projects form part of a larger effort which is aimed at (3) developing a comprehensive and systematic taxonomy of attentional disorders in humans. This latter study will eventually comprise study of patients with cerebral lesions, seizures, dementing diseases, and metabolic illnesses of the brain.

Project DescriptionA. Other Personnel

Connie C. Duncan, Ph.D.	Senior Staff Fellow	LPP, NIMH
Walter H. Kaye, M.D.	Associate Professor	Univ. of Pittsburgh
Richard Coppola, D.Sc.	Senior Engineer Officer	CBD Branch NIMH
Theodore P. Zahn, Ph.D.	Research Psychologist	LPP, NIMH
Roger Porter, M.D.	Chief	EBB, NINCDS
Debbi Fein, Ph.D.	Assistant Professor	Boston Univ.
Daniel R. Weinberger, M.D.	Chief	CBD Branch NIMH

B. Project Description1. Brainstem Mechanisms in Attention Impairment

Current approaches to the neuropsychology of attention impairment have emphasized that the system responsible for the maintenance of attention or consciousness within the brain is most likely represented at a variety of levels of the neuraxis. From an evolutionary point of view, it is clear that the capacity for sustained attentive behavior is present in many species which do not possess more than a rudimentary forebrain or telencephalon. MacLean's analysis of the R-complex within the human brain leads to the view that this "clump of ganglia," which constitutes virtually all of the reptilian brain, can support a variety of ritualistic, repetitive behaviors which could be characterized as sustained and attentive. Evolution progressed and the brain developed additional complexity and volume. Additional capacity for attentive behavior was thus overlaid on the more primitive, although in many aspects thoroughly adequate, brainstem system of the reptile. Therefore, although the system for maintenance of attentive behavior in the human (or higher primate) includes limbic and neocortical components, the brain stem remains a key component and possibly the keystone of the entire system. Authors such as Hughlings Jackson and Penfield and Jasper recognized this in their conceptions, respectively, of "highest-level seizures" and the "centrencephalon." In their theorizing, consciousness was either localized in or regulated by deep brainstem structures. Without reviewing all of the evidence that led to those views of the hierarchical organization of attention and consciousness within the brain, we nevertheless point to the extremely deleterious effects on such capacities of small lesions in the brainstem region of the third and fourth ventricles. In the last ten years, a new technological refinement of evoked-potential methodology has made possible an other-than-theoretical exploration of the role of brainstem structures in certain clinical states. This "far field" or BAER (for brainstem auditory-evoked responses) technique makes it possible to assess the integrity of auditory (and somatosensory) relay nuclei within the brain stem of humans. Although the technique has probably had most utilization in the diagnosis of demyelinating disease, it has also been used in the study of other

neurological and, recently, psychiatric disorders. There may or may not be any specific interest in these sensory systems (auditory, somatosensory) in studying a particular clinical entity (i.e., absence seizures, infantile autism); nevertheless, the possibility of evaluating the functional integrity of certain systems within the brain stem is extraordinarily valuable, and many clinical investigators are using these techniques. We have published work indicating that there are disturbances (prolonged transmission time) in the processing of auditory information in the brain stem in infantile autism and in schizophrenia. We have also shown that in absence seizures (spike-wave activity), both naturally-occurring and experimentally-induced, there may be perturbations of auditory brainstem functioning.

WE have recently completed the analysis of BAERs, recorded in the interictal period, from a group of eight patients with absence epilepsy. These subjects were matched in terms of age, sex and educational level with a group of normal controls. The data indicate that, as in the case of the ictal period in such cases, there are significant prolongations of several of the BAER components in the patients. Thus, significantly longer latencies were seen in components I, II, V and in the transmission time from component III-V. These findings are compatible with the view that more-or-less continuous perturbation of brainstem processes may be occurring in absence epilepsy, and is incompatible with the conception that patients with this disorder are essentially normal between seizure discharges. It should also be noted (section 2 below) that substantial impairment in auditory attention tests were seen in these patients; this is all the more impressive in view of the normal auditory acuity in these subjects and the high-functioning character of a number of the group members. These data are being prepared for publication.

BAERs are now routinely gathered on our patient subjects and we hope within the near future to have publishable quality data from schizophrenic subjects and other clinical populations, as well. We are also planning to gather BAERs in patients with head injuries, as well as possibly recalling some autistic subjects for retesting. BAERs are also included in the alcohol protocol being conducted by Dr. Frances Gabbay, a Guest Researcher from John Hopkins University.

2. Neurobehavioral Studies in Absence Epilepsy

We have for a number of years been studying the absence attack in patients with petit mal/centrencephalic/absence seizures (the terms are more or less interchangeable) as a model state to understand the phenomenon of consciousness/attention. Some of these studies have involved comparing the behavioral capacities of patients suffering from petit mal--as opposed to focal seizure disorders; other studies have involved detailed comparison and contrast between the behavioral and the electroencephalographic symptoms/signs of the disorder. Most recently, these investigations have: (1) used evoked potentials in the visual and auditory modalities as indices of the sensory effects of generalized seizure activity of the symmetrical and synchronous wave and spike variety, and (2) examined changes in the EEG power spectrum prior to WS bursts as prodromal signs which may be used to predict (and

ultimately to control) WS bursts. We propose to continue this line of neurobehavioral investigation, using event-related potentials of various types as well as other behavioral and physiological tools, to refine further our understanding of the nature of altered consciousness in absence (petit mal) epilepsy.

A group of approximately eight subjects with absence epilepsy has now been studied with a full battery of tests, including a complete neuropsychological examination and a number of ERP paradigms requiring varying amounts of attention. Untreated cases with absence epilepsy are difficult to find and persons with good medication control of their seizures are reluctant to serve as subjects. Nevertheless, we now have a sufficiently large group to be able to make some additional contributions to the study of attention in absence epilepsy. Analysis of the neuropsychological data is underway. Preliminary results indicate that although this is a high-functioning group of absence patients, they demonstrate the expected impairment in attention in the interictal period, as assessed by the Continuous Performance Test (CPT). Further, it was found that significantly greater impairment was seen in the auditory version of this task than in the visual version.

Analyses of the ERPs to CPT stimuli revealed impairment in information processing which paralleled the behavioral data. In addition, new insights into the response failures in absence epilepsy were provided by this analysis, which is reported in more detail in protocol Z01 MH 00509-06 LPP.

3. A Taxonomy of Attentional Disorders

The goal of this project is to develop a comprehensive and coherent account of the relation between symptoms of altered or disturbed attention or consciousness as they appear in various clinical entities, the other behavioral and clinical characteristics of the several disorders, and the specific central nervous system damage or disturbance in each disorder. The attentive capacities of the patients are assessed by a number of attention tests including the CPT (continuous performance test), a measure of sustained visual attentive behavior. The ultimate (albeit utopian) goal will be to describe the precise attentive deficit (as opposed to cognitive losses) and the nature of the neuropathophysiology associated with each of the following clinical entities: cerebral lesions (frontal, parietal, temporal lobe, or brainstem); centrencephalic/absence epilepsy; schizophrenia; infantile autism; dementing diseases (Alzheimer's, Korsakoff's, Huntington's); and metabolic diseases (Phenylketonuria, Uremia, Anorexia Nervosa and related illness).

We will attempt, as well, to relate these changes where possible to standardized measures of mnemonic and other cognitive function, and to autonomic indices of attention, arousal, and habituation. Reasonable amounts of data have now been collected on a number of these populations and the work continues.

During the past two years, a theoretical model of the elements of attention has been proposed in a number of publications. This model is based

on a factor analysis of the data from 111 subjects. In addition, it incorporates information from neuroanatomical and neurophysiological sources. It suggests that "attention" comprises a series of behavioral components or elements including the capacities to focus, encode, sustain, shift and execute. Further, it is suggested that those elements are best assessed by different groups of neuropsychological tests (which are incorporated in our LPP test battery). Additionally, it is speculated that these behavioral elements are supported by different regions of the central nervous system.

The elements of a attention model, it is hoped, will provide a useful heuristic device for organizing studies and analyzing data, and will facilitate the development of a taxonomy of attention disorders. The model is discussed further in Z01 MH 00508-06 LPP, which also describes its use in screening for attention disorders in a population of second grade public school children. The model, and the tests on which it is based, have been adopted in studies of the relatives of schizophrenics in County Roscommon, Ireland, and (pending favorable action on grant proposals) by investigators in Seattle studying long-term effects of fetal alcohol syndrome and lead effects on antisocial behavior (University of Pittsburgh). We are also employing the tests and model in our following studies of the high-risk offspring of schizophrenics in Israel (protocol Z01 MH-00471-33 LPP).

Significance to Biomedical Research and to the Program of the Institute

Since attention disturbance is a characteristic of many significant psycho- and neuropathological disorders, it is essential to have a clear empirical and theoretical account of the role and pathophysiological significance of this symptom. Such a theoretical model will aid in understanding the etiology and course of these illnesses and may aid in improving their treatment.

Proposed Course

We have a substantial group of schizophrenic, epileptic, and brain-injured patients tested on our laboratory procedures (i.e., CPT, brainstem auditory-evoked potentials, various tests of cognition and memory, autonomic indices of attention, etc.). We are in the process of preparing the results of these studies for publication.

We have completed an edited book on petit mal epilepsy which is in press, and due to be published in the fall of 1988.

Publications

Mirsky AF, Ray C. Studies in the neuropsychology of attention impairment: human symptoms and animal models. In: Galbraith GC, Kietzman ML, Donchin E, eds. Neurophysiology and psychophysiology: experimental and clinical applications, Hillsdale, NJ: Erlbaum, 1988;114-37.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00504-08 LPP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Models in the Monkey of Generalized Seizures of the Absence Type

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Allan F. Mirsky, Ph.D.	Chief	LPP, NIMH
Others:	Eva Bakay Pragay, Ph.D.	Guest Researcher	Munich, West Germany
	Richard Nakamura, Ph.D.	Guest Researcher	
	Michael Myslobodsky, M.D., Ph.D.	Professor,	Israel
		Univ. of Tel Aviv	CBD Branch
	Richard Coppola, Ph.D.	Engineer	NIMH

COOPERATING UNITS (if any)

Tel-Aviv University, Israel

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.7

PROFESSIONAL:

0.7

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Generalized seizure activity with the electrographic appearance of absence epilepsy (bilaterally symmetrical and synchronous paroxysmal three-per-second spike and wave discharges) can be elicited in the monkey by a variety of methods. These include electrical stimulation of various locations within the brain, injection of convulsant drugs and other substances, and administration of compounds which may alter normal inhibitory mechanisms within the cell. Model seizure states created in these ways are studied in order to test hypotheses about pathophysiological seizure mechanisms, sensory processing and attentional capacities during absence seizures, effects of spike-wave activity on cellular activity, and effects of techniques or maneuvers which may modify or reduce convulsive activity. Most recently this project has involved the following work: we studied the behavioral and electrographic effects of a GABA-enhancer and surveyed the attention-related cells in the frontal lobes of the monkey. Other studies of "attention" cells in inferior parietal and preoccipital cortex have been completed as well.

Project Description

The putative neurotransmitter GABA (γ -aminobutyric acid) is thought to be involved in the central neural processes of inhibition whose perturbation can result in generalized seizure disorders. Two compounds which are metabolically related to GABA are GBL and GHB. γ -butyrolactone (GBL) and the pharmacologically active product of its hydrolytic cleavage, γ -hydroxybutyrate (GHB), produce several central effects of potential significance for therapy and experimental pathology. Winters and Spooner identified GHB effects as epileptogenic or related to "non-convulsant epilepsy." Other research in rodents and monkeys added to the conviction that GHB causes electroencephalographic and behavioral effects akin to petit mal epilepsy.

We attempted to assess the petit mal-like effects induced by GBL by studying the attention-related performance and EEG responses in monkeys administered a single dose of the drug. All animals had been trained to perform a go/no-go visual attention task similar to the Continuous Performance Test (CPT) used in studying human subjects with petit mal. The criterion performance was 80% correct responses.

While there were individual differences in responding after administration of 125 mg/kg of GBL, a dose of 200-250 mg/kg caused a reliable suppression of responding in all subjects. When the testing began 30 minutes following the drug, animals initially responded rapidly and reliably but soon ceased responding altogether. However, they remain sufficiently alert to groom and react to environmental stimuli. Some occasionally resumed responding for several minutes. Offered water, all animals drank it eagerly.

If tested immediately following administration of the drug (200-250 mg/kg), they were able to perform at 60-70% correct; at about 40 minutes the responding came to a complete halt. Here again, monkeys remained competent perceptually and motorically for some time thereafter.

EEG monitored during the task performance showed a build-up of generalized hypersynchronous activity in some areas when performance deficit was noticeable. A pattern resembling 3 cps wave-spike discharges typical of petit mal was never seen either during this period or at the end of the study. These effects are not typical of either petit mal or petit mal status and may be explained by the development of frank sleep. These effects seem related to the general anesthetic properties of GBL (described by some investigators) rather than to its potential (if any) as a model of petit mal.

Significance to Biomedical Research and to the Program of the Institute

This protocol provides information concerning the nature of the attention-support system in the primate brain, the role of various neurotransmitter substances in consciousness and in generalized seizures and contributes to the current efforts to produce an accurate primate-based model of the pathophysiological processes in absence epilepsy.

Proposed Course

The work described here has been accepted for publication in Behavioral Brain Research. Additional findings from this project will be published in the future. We have recently completed a book, which is currently in press, that includes chapters reviewing the recent developments in the biochemistry, electrophysiology and genetics of absence epilepsy. The book will incorporate much of the material germane to this project.

Publications

Mirsky AF, Pragay EB. Brainstem mechanisms in the processing of sensory information: clinical symptoms, animal models, and unit analysis. In: Sheer DE, Pribram KH, eds. Attention: cognitive, brain function and clinical applications; 1988, in press.

Myslobodsky MS, Mirsky AF, Theoretical summary. In: Myslobodsky MS, Mirsky AF, eds. Elements of petit mal epilepsy. New York: Peter Lang, 1988, in press.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00508-06 LPP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropsychological Evaluation of Psychiatric and Neurological Patients

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Connie C. Duncan, Ph.D. Chief, Unit on Psychophysiology

LPP, NIMH

COOPERATING UNITS (if any) BPB, NIMH; MN, NINCDS; LN, NINCDS; Chestnut Lodge Hospital; Johns Hopkins University; Maryland Head Injury Foundation; Division of Neuropsychology, Medical College of Pennsylvania; Department of Psychiatry and Human Genetics, Virginia Commonwealth University.

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.5

PROFESSIONAL:

1.0

OTHER:

1.5

CHECK APPROPRIATE BOXES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A set of comprehensive neuropsychological test batteries is used to provide a complete assessment of various cognitive and sensory functions that can be related to damage or dysfunction in different regions of the brain. The adult battery comprises tests designed to tap the following aspects of behavior: attention, executive functions, language, memory, motor functions, orientation, selected sensory and perceptual functions, vigilance, and visual-spatial functions. In addition, adults are given a test of general intelligence and a personality inventory. In some studies, subjects are administered a structured psychiatric interview. Modified batteries have been developed for the assessment of infants, preschool children, children ages 5-8, and children ages 6-16. The data provided by these batteries are being used to construct neuropsychological profiles of the neurological and psychiatric diagnostic groups under study in the LPP. The LPP has as its major focus disorders involving impaired attention, including schizophrenia, seizures, eating disorders, affective disorders, head injuries, and AIDS dementia complex. Comparisons are being carried out between the neuropsychological profiles of various groups of psychiatric patients and those of patients with known cerebral lesions in specified brain regions. Our data are also being used to delineate neurobehaviorally-defined subgroups within diagnostic categories, an undertaking aimed at reducing variability in psychiatric diagnosis, treatment, and outcome. The data provided by this protocol provide a complete behavioral assessment that may be integrated with concurrently gathered electrophysiological, neuroradiological, and biochemical information.

Project DescriptionA. Other Personnel

Allan F. Mirsky, Ph.D.	Chief	LPP, NIMH
Barbara P. Jones, Ph.D.	Special Expert	LPP, NIMH
Bruno J. Anthony, Ph.D.	Senior Staff Fellow	LPP, NIMH
Loring J. Ingraham, Ph.D.	Senior Staff Fellow	LPP, NIMH
Marie Elliott, M.A.	Psychologist	LPP, NIMH
Edward Turner, M.S.W.	Social Worker	LPP, NIMH
Mary Beth Ahearn, M.A.	Graduate Associate	Department of Mental Hygiene, Johns Hopkins University Chestnut Lodge Hospital
C. Wesley Dingman, M.D.	Assistant Clinical Director	
William W. Eaton, Ph.D.	Associate Professor	Department of Mental Hygiene, Johns Hopkins University
Elkhonon Goldberg, Ph.D.	Director	Division of Neuropsychology, Department of Psychiatry, Medical College of Pennsylvania
Sheppard G. Kellam, M.D.	Chairman	Department of Mental Hygiene, Johns Hopkins University
Kennetn S. Kendler, M.D.	Professor	Department of Psychiatry and Human Genetics, Virignia Commonwealth University
Robert Post, M.D.	Chief	BPB, NIMH
Barry Richmond, M.D.	Senior Surgeon	LN, NINCDS
Carole Robel, B.A.	Project Coordinator	Maryland Head Injury Foundation
William H. Theodore, M.D.	Acting Chief	CES, MNB, NINCDS

B. Objectives

This project has as its goal the investigation of neurobehavioral functioning in neuropsychiatric patients, such that: (1) Neuropsychological profiles of diagnostically distinct groups can be obtained, and differences in the profiles among groups can be used as an indication of specific organic influences in the psychopathology of these patient groups; (2) Neuro-psychological profiles of patients within a heterogeneous diagnostic classification can be obtained. Differences between patients can provide neurobehaviorally-defined subgroups that might reduce variability in diagnosis

and treatment, as well as improve outcome; and (3) The obtained comprehensive neurobehavioral data can be correlated with electrophysiological, biochemical, and neuroradiological data that are being collected concurrently on these patients to provide a link between pathophysiology and behavior.

C. Methods Employed

The standard adult neuropsychological battery, used for many of our studies, is presented below. Cognitive and sensory functions are presented in tabular form along with the test(s) used to assess them. Modified batteries have been assembled for some of our studies, and we have assembled special batteries for the assessment of children in three different age groups from 2 to 16. Administration of the adult battery takes 8-12 hours and occurs over several days. Administration of the infant and child batteries takes from 1/2 hour to 6 hours, depending on the age of the child, and is divided into as many sessions as needed to avoid overtaxing the subject.

LPP Adult Neuropsychological Test Battery

<u>FUNCTION</u>	<u>TEST</u>
<u>Executive</u>	
Sequencing, Attention	Trail Making Test
Attention	Stroop Color-Word Interference Test
	Letter Cancellation Test
Perception and Reasoning	Raven Standard Progressive Matrices
Concept Formation and Abstraction	Wisconsin Card Sorting Test; Category Test (Halstead)
<u>General Intelligence</u>	Wechsler Adult Intelligence Scale-Revised
<u>Language</u>	
Initiation	Controlled Oral Word Association Test
Lexical	Boston Naming Test
Written	Boston Diagnostic Aphasia Examination--Narrative Writing
Comprehension	Token Test (Spreen & Benton)
	Semantic Aphasia Test (Goldberg)
Auditory Discrimination	Wepman Auditory Discrimination Test
<u>Memory</u>	
Global	Wechsler Memory Scale I
Recent Verbal Memory	Buschke Selective Reminding Test
	Rey Auditory Verbal Learning Test
Recent Visual-Spatial Memory	Recurring Figures Test (Kimura)

LPP Adult Neuropsychological Battery (continued)

Remote Memory	Complex Figure Test (Rey-Osterrieth) Boston Famous Faces Test (Short Form) Television Test Boston Recall Test (Short Form)
<u>Motor Functions</u>	Purdue Pegboard Test Boston Apraxia Test
<u>Orientation</u>	Temporal Orientation Test (Benton)
<u>Personality</u>	Minnesota Multiphasic Personality Inventory
<u>Sensory and Perceptual</u>	Bioptr Vision Tests Dvorine Pseudo-isochromatic Plates Titmus Stereo Tests Harris Test of Hand Dominance Eye Dominance Test
<u>Vigilance</u>	Continuous Performance Test (CPT)
<u>Visual-Spatial</u>	Hooper Visual Organization Test Embedded Figures Test (Witkin) Butters' Embedded Figures Test

D. Major Findings

The test battery or parts thereof has been administered to a total of 346 subjects in our laboratory (130 males, 216 females), of which 79 were normal control subjects (26 males, 53 females). In addition, 114 followup test sessions have been conducted in our laboratory for protocols involving two or more serial neuropsychological evaluations. Outside the laboratory, 485 subjects have been tested in a collaborative study with the Johns Hopkins School of Hygiene and Public Health. The total number of subjects tested, both within and outside of the laboratory and including followup testing, is 945.

1. Eating Disorders

The first phase of the eating disorders research, an investigation of cognition and personality in these disorders, has now been completed. The major portion of this study employed a cross-sectional design comparing the test performances of four groups of women (28 underweight anorexics, 41 normal-weight bulimics, 15 weight-restored anorexics, and 38 normal controls) on a comprehensive neuropsychological test battery and the Minnesota Multiphasic Personality Inventory (MMPI). In addition, the study included a longitudinal comparison of 9 underweight anorexics and 10 normal controls who returned for followup testing after an average interval of 3.7 (± 1.2) months

for the anorexics and 5.9 (± 3.9) months for the controls.

In the cross-sectional comparison we found an association between acute eating disorders (anorexia and bulimia) and decrements on a number of cognitive and personality variables, specifically several measures related to attentional capacities (Revised Letter Cancellation Test and WAIS-R Arithmetic), verbal learning (Babcock Story Recall Test, immediate recall), spatial reasoning (Raven Standard Progressive Matrices), and several clinical scales on the MMPI (1 [Hypochondriasis], 2 [Depression], 4 [Psychopathic Deviate], 6 [Paranoia], 7 [Psychasthenia], and 8 [Schizophrenia]). The underweight anorexics were the most impaired of the eating disorder groups on both cognitive and personality measures; in addition to the measures noted above, they were impaired relative to normal controls on MMPI Hysteria Scale; WAIS-R Full Scale and Verbal IQ, Vocabulary, Information, and Block Design; Wechsler Memory Scale Logical Memory; the Recurring Figures Test; and the Purdue Pegboard Test. Somewhat to our surprise, even the long-term (i.e., six months) weight-restored anorexics obtained abnormal scores relative to normal controls on several indices: two attentional measures (from the Continuous Performance Test), measures of verbal and nonverbal learning and retention (Babcock Story Recall Test immediate recall, Recurring Figures Test), and spatial reasoning (Embedded Figures, time). In order to assess the relationship between the demonstrated impairments of attention in the eating disorders groups and the impairments of other cognitive functions, we used analyses of covariance based on previous factor analyses of attentional measures in the LPP. Controlling for the effects of attention in this way resulted in a loss of a significant group main effect for all of the cognitive measures noted above except the Recurring Figures Test and the Purdue Pegboard Test. The results thus suggest a major deficit in attention in patients with eating disorders, as well in the visual-spatial abilities tapped by the Recurring Figures Test.

Longitudinal comparisons have yielded some puzzling findings: Underweight anorexics who returned for followup testing 3.7 months after weight restoration were considerably more impaired on the cognitive measures (compared to normal controls) than were the weight-restored anorexics in our cross-sectional comparison (mean 6 months past weight restoration). Possibly the confounding effects of starvation itself were more apparent in the 3.7 month restored group, since they had been weight-restored, on the average, for 2 months less than the long-term weight-restored groups. However, a University of Minnesota study of the effects of human starvation on intellectual and personality functioning produced relatively minor findings. These results argue against the conclusion that time since starvation per se produced the effects we saw. More likely, some sampling bias may have been operating, in that anorexics who were able to maintain their restored weight for at least 6 months represent a less severely disordered group of patients. It is well known that from a statistical point of view, anorexia carries a poor prognosis.

The results of this study are seen as confirming and extending the findings of previous investigations, which have demonstrated impairments of attention and right hemisphere functioning in eating disorders. The theory of

relative right-hemisphere dominance for vigilance, arousal, and directed attention may be relevant here. That is, it may be possible to explain the consistent findings of attentional deficits and right-hemisphere impairments in patients with eating disorders on the basis of functional impairment of the right hemisphere. One might also speculate about an abnormality involving the locus ceruleus of the reticular activating system, which is known to be involved in attention and is also known to be rich in noradrenergic receptors. The latter hypothesis is rendered more plausible by previous findings of state-independent abnormalities of norepinephrine metabolism in weight-restored anorexic patients.

We are currently in the process of concluding the eating disorders research with a study focusing on the differences between the two types of anorexic patients, namely, restrictors, who control their weight by abstaining from food, and bulimarexics, who control their weight by vomiting and/or laxative abuse. For the purposes of this study, we are currently testing additional weight-restored anorexics of both types.

2. Affective Disorders

In a study of patients with bipolar affective disorder, we set out not only to delineate a neuropsychological profile for patients with bipolar affective disorder but also to compare the neuropsychological profile of this group with that of patients with complex partial seizures. Recent developments in the study of affective disorders and their treatments have suggested a rationale for examining the similarities and differences between patients with bipolar affective illness and patients with complex partial seizures. Post, Ballenger, and their colleagues have proposed a kindling model of affective illness and temporal lobe epilepsy. Briefly, it is postulated that repeated seizures (in the case of temporal lobe epilepsy) or repeated biochemical and/or psychological stresses (in the case of affective illness) produce cumulative bioelectrical changes, which in turn result in abnormal limbic neuronal sensitization and major psychiatric disturbances. Both disorders are thought to involve temporal lobe and limbic system abnormalities, and both disorders have been shown to respond to treatment with anticonvulsant agents, including carbamazepine.

To date, we have tested 16 patients with bipolar affective disorder, 5 patients with complex partial seizures, and 22 normal controls. A shortened version of the LPP's neuropsychological battery was administered in this study in order to lessen the demands of the testing on the bipolar patients, some of whom were seriously depressed. When we analyzed the data collected thus far, we found that the complex partial seizure patients performed significantly worse than either the bipolar patients or the normal controls on a number of measures including those of general intellectual functioning (WAIS-R Full Scale, Verbal, and Performance IQ), attention (Trail Making Test, Letter Cancellation Test, Stroop word, color, and color-word scores), learning and retention (Buschke Selective Reminding Procedure; Babcock Story Recall Test, percent error), verbal fluency (Controlled Word Association Test), and motor speed (Purdue Pegboard Test). Bipolar affective disorder patient performed significantly worse than normal controls only on measures of motor speed as

reflected in WAIS-R Performance IQ and the Purdue Pegboard Test.

The group differences in Full Scale IQ were substantial. This finding may well reflect a bona fide difference between bipolar affective patients and complex partial seizure patients in that seizures may have a deleterious effect on intellectual functioning; however, the question remains unanswered as to whether more specific differences in cognitive functioning between these two groups may be found. We believe that the most productive approach to this question is to test additional high-functioning complex partial seizure patients so that the Full Scale IQs of these two groups may be more nearly equivalent. In the meantime, we approached this question by statistically controlling for the effects of differences in overall level of intellectual functioning: Analyses of covariance were performed on all of the measures used in this study which had yielded group differences. These analyses showed that even after controlling for differences in overall intelligence, complex partial seizure patients performed worse than either normal controls or bipolar affective disorder patients on some measures of verbal learning and retention (Buschke Selective Reminding Procedure, recall and consistency scores), motor speed (Purdue Pegboard Test), and attention (Letter Cancellation Test: letters surveyed, correct cancellations; Trail Making Test; Stroop word and color-word scores) and that they performed worse than normal controls on one of the attentional measures (Stroop color score); while, on the other hand, bipolar affective disorder patients performed worse than normal controls only on the Purdue Pegboard Test.

Our preliminary conclusions from this work may be summarized as follows: (1) The neuropsychological profiles of bipolar affective disorder patients and complex partial seizure patients are similar in showing lowered WAIS-R Performance IQ and slowed performance on the Purdue Pegboard Test relative to normal controls; and (2) The neuropsychological profiles of bipolar affective disorder patients and complex partial seizure patients differ in that the latter show impairments on a much broader range of cognitive functions. These impairments appear in such functions as attention, learning, verbal fluency, and retention and seem to be suggestive of a greater degree of compromise in frontal-limbic systems subserving important aspects of cognition.

3. The Elements of Attention--A Working Model

It was within the context of attempting to analyze the nature of the disturbed attention in schizophrenia that Zubin proposed in 1975 a view of the "elements" of attention that has proven useful in interpreting the results of our own research. According to Zubin's analysis, attention can be subdivided into a number of elements or aspects. These include (a) the capacity to focus upon or select some part of the environment, (b) the ability to sustain or maintain that focus for an appreciable period, and (c) the ability to shift adaptively from one aspect or element of the environment to another.

This analysis of the elements of attention accords well with some recent data obtained in our laboratory. These data are derived from an analysis of an extensive series of neuropsychological tests administered to a variety of neuropsychiatric patients and normal controls, studied either as inpatients or

outpatients under a variety of research protocols. The data of most interest for this discussion involve the factor analysis of 10 test scores commonly considered to be measures of attention which are, in turn, derived from eight frequently used tests of attention. The tests were as follows:

1. The Trail Making Test
2. Talland Letter Cancellation Test
3. Digit Symbol Substitution Test (DSST), a subtest of the Wechsler Adult Intelligence Scale-Revised (WAIS-R)
4. Stroop Color-Word Interference Test
5. Continuous Performance Test (CPT)--Mean number of correct responses, X and AX tasks combined.
6. CPT--Mean number of errors of commission, A and AX tasks
7. CPT--Mean reaction time for correct responses, X and AX tasks combined
8. Digit Span (a subtest of the WAIS-R)
9. Arithmetic (a subtest of the WAIS-R)
10. Wisconsin Card Sorting Test (WCST)

The scores obtained by a group of 111 adult subjects (who received a complete battery of neuropsychological tests) were subjected to a factor analysis using an orthogonal rotation. The results of this factor analysis indicated that the 10 scores described above could be characterized by four factors that accounted for over 71% of the variance.

Four tests loaded heavily on Factor 1; they are Trail Making, Talland Letter Cancellation, DSST, and Stroop. They all appear to tap some aspect of perceptual-motor speed and could be designated as measuring the focusing aspect of attention. Since speed is in fact a key feature of performance on each of these four tests, the execute component of the task seems intertwined with focus in this factor. The CPT measures alone load heavily on Factor 2, which can unambiguously be designated a vigilance factor; therefore, it seems reasonable to label this as reflecting the sustain component of attention. Factor 3 is represented only by the Digit Span and Arithmetic tests. It seems to assess a numerical-mnemonic quality of attention; the encode aspect of attention/information processing seems to capture this. Finally, Factor 4 is represented by only one test, the WCST. As in the case of Factor 2, the identity of this factor seems readily apparent; it taps the flexibility aspect of attention--the capacity to shift.

This factor analysis-derived elements of attention model has proven very useful in the interpretation and the design of research in our laboratory and in studies being conducted with collaborators outside of NIMH

4. Childhood Attentional Disorders

Our goal in this project is to develop a neuropsychologically-based taxonomy of attention in young children. A major problem in examining attention disturbance in young children has been an oversimplified, unitary concept of attention. Therefore, we constructed an Attention Battery, based in part on our research on attention in adults, designed to tap different

processes or constructs underlying attentive behavior. The battery includes three versions of the X Task of the CPT (standard, using auditory distraction, using degraded visual stimuli), Digit Cancellation (with and without auditory distraction), the Wisconsin Card Sorting Test, subtests of the WISC-R, the Peabody Picture Vocabulary Test, and an abbreviated examination of soft neurological signs.

Investigations employing this battery are proceeding along two lines. In the first, we are attempting to understand the latent structure of attention in young children, its developmental course, and the relationship between disturbances in attention and present and future maladaptive behavior and learning. So far, this effort has involved administration of the Attention Battery to a large, population-based sample of young children through a collaborative effort with the Prevention Research Center (PRC). The PRC, a joint project of the Baltimore City Public School System and the Department of Mental Hygiene of the Johns Hopkins School of Hygiene, examines critical developmental pathways and also interventions designed to reduce psychopathology and substance abuse. Two cohorts (approximately 2400 children) of first-grade children attending 19 elementary schools in East Baltimore are being assessed periodically through teacher ratings, peer nominations, independent behavior time sampling, and structured self-reports coupled with information on school progress.

Over the past year, we have scored, edited, coded, and begun to analyze Attention Battery data from a representative sample of 435 second graders participating in the PRC project. In order to examine objectively how different measures in the battery cluster to reflect underlying constructs, we intend to employ confirmatory factor analysis which uses linear structural equation modeling (LISREL) to evaluate alternative models for the framework. We recently completed a standard factor analysis on the data of 428 children and discovered essentially the same four factors that are present in the adult data (section 3): focus, execute; sustain; encode; shift. Some differences emerged between the results of analyses applied to the boys' as opposed to the girls' data; a separate, fifth, speed factor is present in the boys that is not evident in the girls. In subsequent analyses, we intend to related factor scores to social adaptation, psychiatric symptomology, and school achievement.

In a second line of investigation, the battery is being used to examine the nature of attentive deficits in groups of children with clinically diagnosed attention deficits. The correlation between Attention Deficit Disorder with Hyperactivity (ADHD) and the later development of substance abuse, delinquency, and various forms of psychopathology has been well documented in the literature. However, questions persist as to whether the attentional disturbance that characterizes children with ADHD can be distinguished from those associated with other neuropsychiatric disorders of childhood. Characteristics such as "doesn't listen" or "easily distracted" may have different clinical significance when they are not associated with hyperactivity. Thus, the Attention Battery is being administered to 7- to 11-year-old children with (ADHD), and to children with Tourette's Disorder

(TD) who also exhibit significant difficulties with attention. So far, we have tested 13 children with ADHD and 3 children with TD and attention difficulties. These children are participating in a comprehensive evaluation involving measurement of autonomic and brain event-related potential indices of regulatory difficulties.

5. Gaucher's Disease

We have completed a small study of neuropsychological functioning in children and adolescents with Gaucher's Disease, Type I or III. Gaucher's Disease is a rare, autosomal recessive disorder caused by a deficiency of glucocerebrosidase and the resulting accumulation of glucocerebroside in organs and tissues. Clinically, three forms are distinguished: Type I, usually characterized as a nonneuropathic form in which the central nervous system (CNS) is not affected (although CNS involvement has been reported in a small number of patients); Type II, an acute neuronopathic, infantile form, usually apparent before 6 months of age and fatal by 2 years of age; and Type III, a subacute neuronopathic, juvenile form with some CNS involvement. The precise nature of the neuropathological changes in Type III has not been well documented.

We studied 6 patients with Type I Gaucher's Disease (age range 5 years to 14 years) and 6 patients with Type III Gaucher's Disease (age range 7 years to 14 years). Because of the demands of other clinical studies at NIH, patients received only the Wechsler Intelligence Scale for Children-Revised (WISC-R) and the Continuous Performance Test (CPT), a sensitive test of sustained attention. Although there were substantial differences between the two groups in Full Scale (112.1 versus 96.8), Verbal (108.8 versus 98.7), and Performance IQ (113.7 versus 96.3), the difference in Performance IQ was the only one to attain statistical significance (Type I score listed first in each pair). Analyses of the WISC-R subtest scores revealed that the groups differed significantly on Arithmetic (10.8 versus 8.8) and Block Design (11.8 versus 7.7), although the Type III patients performed worse than the Type I patients on all 11 subtests. On the CPT there were no significant differences on any of 6 different performance measures.

Although our data are limited, they appear to confirm the clinical descriptions of minimal CNS involvement in Type I Gaucher's Disease and some significant CNS involvement in Type III Gaucher's Disease. Because Type III patients were impaired relative to Type I patients on two WISC-R subtests and on Performance IQ but not on the CPT, we suspect that some cortical involvement but an intact subcortical activating system may characterize Type III patients, at least through childhood and adolescence. While arithmetic and spatial tasks such as Block Design and Performance IQ are all thought to reflect aspects of right-hemisphere functioning, in the case of a systemic disease such as Gaucher's Disease, this pattern of findings would appear to reflect an early onset of a diffuse process rather than selective involvement of the right hemisphere.

6. Schizophrenia and Closed Head Injuries

Our neuropsychological approach to schizophrenia differs from that of most investigators in its emphasis on attention, executive functions, and related measures. Because it has been hypothesized that the site of dysfunction in schizophrenia is primarily in bilateral frontotemporal regions, we are comparing the neuropsychological profile of a group of hospitalized chronic schizophrenic patients to that of a group of closed head-injury patients with bilateral frontal or bilateral frontotemporal damage. At present we have tested 9 hospitalized chronic schizophrenics, 4 patients with bilateral frontal or bilateral frontotemporal lesions, and 13 normal controls. We are hoping to increase the sizes of both the schizophrenic and bilateral frontotemporal patient groups. In comparison with the standard LPP neuropsychological test battery, the battery we have used in this study contains more measures of executive functions, which are critical in the assessment of frontal lobe functioning.

Preliminary analyses of our relatively small samples reveal that, somewhat surprisingly, our chronic schizophrenic patients performed significantly worse than both comparison groups on a number of measures. The measures on which schizophrenics performed worse than the bilateral frontotemporal patients or normal controls included Full Scale and Performance IQ, attention (four measures from the CPT), naming, verbal learning and retention, motor speed, and set formation and set shifting. These results are consistent with those of previous studies showing impairments of attention, learning, motor speed, and some aspects of left-hemisphere functioning in schizophrenic patients. The patients with bilateral frontal or bilateral frontotemporal lesions performed significantly worse than the normal controls on two measures from the Continuous Performance Test. However, as in the affective disorder study, we found ourselves faced with the possible confounding of effects of general level of intellectual functioning with those of disease entity. Although we hope to be able to bring the IQ levels a little closer as we expand our patient groups, we again performed some analyses of covariance in order to see what group effects remained after the effects of overall IQ level had been statistically controlled. The analyses of covariance revealed that when the effect of overall IQ level had thus been taken into account, schizophrenics still performed worse than either bilateral frontotemporal lesion patients or normal controls on measures of attention (vigilance), set shifting, verbal learning and retention, and naming (word finding).

Our preliminary conclusions from this study may be stated as follows: (1) The neuropsychological profiles of schizophrenic patients and closed head-injury patients with bilateral frontotemporal lesions are similar in showing impairments relative to normal controls on a sensitive measure of vigilance, the CPT; (2) Schizophrenic patients are differentially impaired relative to both normal controls and bilateral frontotemporal patients on measures of verbal learning and retention, word finding, set formation, and set shifting. These findings implicate the same locus of dysfunction, namely, frontal and temporal lobes, for both of the patient groups, but suggest a greater degree of dysfunction in the schizophrenics in this sample and also differential involvement of the left frontal and temporal regions.

7. Schizophrenia: The Genetic Epidemiology of Schizophrenia in Ireland

The LPP is collaborating with Dr. Kenneth S. Kendler of the Department of Psychiatry and Human Genetics at the Medical College of Virginia in a study of the genetic epidemiology of schizophrenia in Ireland. The western part of Ireland is an ideal place for such a study as it has one of the highest treated prevalence rates (and presumably one of the highest incidence rates) of schizophrenia in the world. Using the Roscommon County Psychiatric Case Register, 250 schizophrenic probands and 100 affective disorder probands are being identified and asked to participate in the study. Approximately 150 control probands are being selected from the Irish electoral rolls. From those who agree to participate, a list of all living first-degree relatives will be obtained. The research staff in Ireland will then evaluate as many first-degree relatives as possible on a number of measures including portions of several standardized psychiatric interviews, a family history questionnaire, several scales assessing schizotypal signs and symptoms, an objective personality questionnaire, eye tracking, and neuropsychological measures of attention. Impaired attention, deficient smooth pursuit in eye movement, and schizotypal signs and symptoms are all thought to be possible markers for the genetic vulnerability to schizophrenia.

For this collaborative study, the LPP has been asked to select an optimal battery of neuropsychological measures of attention, to train the Irish research staff in the administration and scoring of the battery, and to analyze and report the results from this portion of the study. Among neuropsychological laboratories, the LPP has performed the most extensive research on attention and its disorders, including impairments of attention in schizophrenic patients and in children at risk for the development of schizophrenia. We will be using an attention battery developed in the LPP and tested in a variety of patient populations over the past few years.

Thus far, we have designed the battery and trained the Irish research staff in its administration and scoring. Practice testing has nearly been completed, and the staff will soon begin collecting pilot data.

8. Schizophrenia: A Twin Study of Putative Indices of the Genetic Vulnerability to Schizophrenia

The LPP is collaborating with Dr. Kenneth S. Kendler in a twin study investigating putative indices of the genetic vulnerability to schizophrenia. This study will examine correlations for several supposed indices or markers of the genetic vulnerability to schizophrenia in monozygotic and dizygotic twins, and the patterning of these indices relative to one another. One goal of this research is to examine models for the genetic transmission of schizophrenia. In the study's initial phase, 50 to 60 pairs of twins from the Virginia Twin Registry established at the Medical College of Virginia will be examined using four types of measures: neuropsychological measures of attention, eye tracking, an interview-based assessment of schizotypal signs and symptoms, and a self-report questionnaire assessment of schizotypal signs and symptoms. Impaired attention, deficient smoothness in eye tracking, and schizotypal signs and symptoms are all thought to be possible markers for the

genetic vulnerability to schizophrenia.

The LPP's role in this collaborative study is to select the neuro-psychological measures of attention, to train Medical College of Virginia personnel in their administration and scoring, and to analyze and interpret the results on neuropsychological measures of attention. We are using an attention battery that was developed in the LPP and has been in use for the past several years. Recent LPP factor analytic studies have suggested four independent factors of attention that are assessed in the battery of 10 attentional measures: focus-execute, sustain, encode, shift. The results of this study should help further knowledge about genetic factors in impaired attention, about impaired attention as a marker for the inherited vulnerability to schizophrenia, and about those particular aspects of attention which might be deficient in those persons with an inherited vulnerability to schizophrenia.

9. AIDS Dementia Complex

The LPP has responded to the recent discovery of the neurotropic and neurotoxic aspects of HIV infection by investigating the application and utility of the LPP Adult Neuropsychological Test Battery for the detection and measurement of cognitive changes associated with HIV infection. A review of the literature on assessment methodologies that have demonstrated empirical utility in measuring cognitive changes early in the course of HIV infection, as well as in the more severe symptoms associated with AIDS Dementia Complex, shows that the LPP battery includes the instruments which have proven most sensitive in studies of cognitive changes associated with HIV infection. In addition, the LPP battery contains particularly sensitive tests of attention that may enable earlier detection of HIV-related changes.

The LPP will continue to be involved in the early assessment and prediction of HIV-related cognitive changes as we refine the use of our battery with this population. In particular, we believe the application of the LPP's approach to integrating psychophysiological and neuropsychological assessment techniques with its expertise in longitudinal studies will prove fruitful in detecting and understanding the consequences of the neurotropic aspects of HIV infection.

Proposed Course

In the coming year, we expect to be able to complete the following studies with the testing of additional experimental subjects and controls: studies on eating disorders (restrictor anorexics versus bulimarexics), bipolar affective disorder versus complex partial seizures, and schizophrenia versus bilateral frontotemporal lesions. We are planning to increase our sample sizes for several of the other, ongoing studies, in order to provide greater power to our analyses. These studies include those of normal children and children at risk for the later development of substance abuse, delinquency, and/or psychopathology in the LPP-Johns Hopkins collaborative study; the genetic epidemiology of schizophrenia in Ireland; putative indices of vulnerability to schizophrenia; and differential effects on cognitive functioning of different

types of closed head injuries. In addition, we plan to increase the number of normal controls assessed using all forms of the neuropsychological batteries to facilitate comparison with the various patient groups. A number of normal controls will also receive followup neuropsychological testing in order to allow for the analysis of data in studies that involve repeated neuropsychological testing and to assess the stability of the measures. We expect to expand our investigation of HIV related cognitive changes using the LPP Adult Neuropsychological Test Battery. During the coming year, we plan to begin a more intensive study of the correlations between the neuropsychological test data and electrophysiological data that are gathered concurrently on many of these patient populations as well as the relationship to other biological measures.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00509-06 LPP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Attention Disorders As Assessed by Brain Event-Related Potentials

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Connie C. Duncan, Ph.D., Chief, Unit on Psychophysiology, LPP, NIMH

COOPERATING UNITS (if any) Clinical Psychobiology Branch, Neuropsychiatry Branch, Child Psychiatry Branch, Laboratory of Clinical Science, NIMH; Chestnut Lodge Hospital; Medical Neurology Branch, NINCDS; Johns Hopkins University; Maryland Head Injury Foundation.

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.2

PROFESSIONAL:

1.0

OTHER:

2.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this project is to investigate the roles of brain event-related potentials, attention, and information processing and their interrelationships in the etiology, pathology, and prognosis of psychiatric and neurological disorders. Major emphasis is on the diagnostic specificity of disorders of attention and cognition and the identification of the specific aspects or stages of information processing underlying observed decrements in performance. Concurrently recorded brain event-related potentials and performance on cognitive tasks are used to differentiate patterns of dysfunction in attentive mechanisms in subjects with diagnoses of seasonal affective disorder, schizophrenia, seizures, eating disorders, dyslexia, closed head injury, attention deficit with hyperactivity disorder, and Tourette's Disorder. A related objective of this project is to differentiate state versus trait attributes of these disorders to increase understanding of their etiologies. Brain event-related potentials are also used to investigate the role of altered neurochemical mechanisms by comparing drug-induced electrophysiological and behavioral effects with those seen in the various disorders. Psychological correlates are investigated by relating the data to extensive neuropsychological, psychiatric, and personality measures as well as to performance on behavioral tasks.

Project DescriptionA. Other Personnel

Allan F. Mirsky, Ph.D.	Chief	LPP, NIMH
Bruno J. Anthony, Ph.D.	Senior Staff Fellow	LPP, NIMH
Barbara P. Jones, Ph.D.	Special Expert	LPP, NIMH
Edward Turner, M.S.W.	Research Social Worker	LPP, NIMH
Norman E. Rosenthal, M.D.	Chief, Unit of Out-Patient Studies	CPB, NIMH
Robert G. Skwerer, M.D.	Medical Staff Fellow	CPB, NIMH
Darrell G. Kirch, M.D.	Associate Clinical Director	NPB, NIMH
Michael F. Egan, M.D.	Medical Staff Fellow	NPB, NIMH
Judith M. Rumsey, Ph.D.	Research Psychologist	CPB, NIMH
Dennis L. Murphy, M.D.	Chief	LCS, NIMH
C. Wesley Dingman, M.D.	Assistant Clinical Director	Chestnut Lodge Hospital
William H. Theodore, M.D.	Acting Chief	CES, MNB, NINCDS
Martha B. Denckla, M.D.	Professor	Department of Neurology, Johns Hopkins University
Carole Robel, B.A.	Project Coordinator	Maryland Head Injury Foundation

B. Objectives

The major objective of this project is to yield data that will illuminate the neurophysiological bases of the cognitive and attentional deficits in the clinical disorders of seasonal affective disorder, schizophrenia, epilepsy, eating disorders, dyslexia, closed head injury, attention deficit with hyperactivity, Tourette's Disorder, and other forms of brain pathology. Defining the specific ways in which information processing can fail may provide new diagnostic strategies for more effective evaluation and treatment of patients with attentional and cognitive impairments. A related objective of this project is to differentiate state versus trait attributes of these disorders to increase understanding of their etiologies. Concurrently obtained brain event-related potentials (ERPs) and measures of performance during active cognitive processing are used to define the mechanisms of attention failure in these syndromes. Defining and understanding the different determinants and forms of attentional and cognitive failure is diagnostically important as well as useful in characterizing the psychobiology of attention disorders. Finding differences in overall response levels between normal subjects and patients on our electrophysiological and behavioral measures is a necessary first step; however, the goal is to use the knowledge to lead to new approaches to classification and treatment, to increased understanding of etiology, and, eventually, to effective preventive interventions.

C. Methods Employed

1. Electrophysiological Assessment

The general methods of these studies include recording the EEG with nonpolarizable electrodes from frontal (FPz, Fz, F3, F4), central (Cz, C3, C4), parietal (Pz, P3, P4), and occipital (Oz) scalp sites according to the International 10/20 system, all referred to linked earlobes. Stimulus presentation and data collection are controlled by a PDP-11/34 or PDP-11/73 computer. The electrooculogram is recorded between supraorbital and outer canthal positions of the left eye. Trials with eye movement artifacts are either discarded or the EEG is corrected mathematically. The EEG is averaged to yield ERPs. Since these scalp-recorded electrical waves are associated in time with either an event in the environment, such as the presentation of a stimulus, or with an internal cognitive event, they are called brain event-related potentials.

Brainstem auditory evoked responses (BAERs) are used to measure, directly and noninvasively, the progress of a sensory signal through brainstem to the cortex, and thereby obtain a measure of the integrity of brainstem functioning. BAERs are obtained by presenting click stimuli to the ears.

Evaluation of endogenous components of the ERP, associated with higher-level processes such as selective attention, learning, and memory, yields information on the attentional and cognitive functioning of the subject. The ERPs are elicited by trains of auditory or visual stimuli presented in the context of an attentional or cognitive task. The selection of discrimination and memory tasks allows for the measurement of a pattern of cognitive behaviors and associated ERPs, where different components reflect different aspects of information processing. Using ERPs, it is possible to get an indication of the subject's processing of all environmental stimuli, both relevant and irrelevant, and thus to assess, for example, the differential processing that is the hallmark of selective attention.

A major focus of our investigations is the "P300" component of the ERP. The P300 is a manifestation of the cognitive activity invoked by a task-relevant stimulus. This scalp-derived electrical potential appears 300 msec or longer after an event and is a positive voltage as recorded on the scalp; hence the name P300. The amplitude of the P300 component varies in direct proportion to the attentional resources allocated to process a stimulus. It is also a sensitive indicator of orienting reactions to novel, surprising, or incongruous stimuli and a predictor of the memorability of events. Moreover, P300 allows a direct evaluation of the subjective probabilities that a subject assigns to event outcomes and may reflect the extent to which a stimulus is encoded. The latency of P300 has been shown to index the duration of perceptual and cognitive processing involved in evaluating a stimulus and to be independent of the time involved in response production. ERPs can thus help to clarify the timing and order of neural events in information processing activities and to identify the aspects or stages of information processing responsible for observed decrements on

cognitive tasks in a variety of clinical populations.

2. Neuropsychological Assessment

Normal volunteers are screened by a psychologist who uses the lifetime version of the Schedule for Affective Disorders and Schizophrenia (SADS-L) to exclude those with past or current psychopathology or with first-degree relatives with such a history. Many patients and normal volunteers are evaluated on an extensive neuropsychological battery of cognitive and sensory functioning. When appropriate, tests of formal thought disorder are administered. We plan to correlate the neuropsychological and electrophysiological data to aid in the classification of disorders characterized by attentional deficit and cognitive failure.

3. Biological Assessment

In collaboration with other laboratories, patients are assessed for treatment responsiveness. In some studies, blood, urine, and/or cerebrospinal fluid measurements reflecting neurochemical activity are correlated with electrophysiological, neuropsychological, and behavioral data. We also plan to use magnetic resonance imaging (MRI scan) as a measure of ventricular size. These data will be correlated with electrophysiological data to yield information on the relation between ERPs and cerebral structures.

D. Major Findings

We are using a variety of attentional paradigms, which tap visual and auditory information processing systems, to investigate patients with seasonal affective disorder, schizophrenia, absence epilepsy, eating disorders (anorexia nervosa and bulimia), adult dyslexia, closed head injury, attention deficit with hyperactivity disorder, and Tourette's Disorder. These tests provide a differential assessment of specific types of attention, including the ability to initiate, select, inhibit, shift, and sustain attention. The protocol also includes evaluation of automatic and controlled cognitive processes. The rationale for the approach of using the same paradigms, which tap specific cognitive processes, on different patient groups is to allow inferences about which processes are uniquely impaired in one group in comparison with other groups. To determine whether ERPs can serve as sensitive yet specific markers of disorder, patients with diverse symptomatology and diagnoses are compared. The ERP measures are correlated with concurrently recorded behavioral responses, including reaction time, and in some studies, with performance on neuropsychological tests.

A number of studies have been completed during the past year, and the results are now either in press or are being analyzed in preparation for publication. The studies include investigations of seasonal affective disorder, schizophrenia, absence epilepsy, eating disorders, the alpha-2 adrenergic agonist clonidine, and dyslexia. The results of each of these studies are summarized briefly below.

1. Seasonal Affective Disorder

Seasonal affective disorder (SAD) is a syndrome characterized by recurrent major depressions during the fall and winter months, when daylight is at a minimum, alternating with spontaneous remissions as daylight increases in the spring. The winter symptoms of SAD include sadness, anxiety, decreased activity, impaired ability to concentrate, increased appetite and weight, and hypersomnia. The most salient characteristic of the depression is its sensitivity to changes in environmental light. The antidepressant effects of bright artificial light (or "phototherapy") have been documented extensively.

We used the P300 component of the ERP to probe the dynamic changes in brain function that accompany phototherapy in SAD patients. The clinical response to phototherapy in patients with SAD was found to be highly correlated with increased visual attention, as indexed by enhanced P300. The enhancement of P300 was evident within the first two days of treatment and increased monotonically during the period of phototherapy. No comparable changes were seen in the P300s elicited by stimuli in the auditory modality.

Seventeen patients who met the criteria for SAD and 11 matched normal controls were tested after at least nine days of phototherapy (2500 lux, full-spectrum fluorescent lights, 2.5 hours in the a.m. and p.m.) ("On Lights"). ERP testing followed immediately after the morning phototherapy, at 10 a.m. Subjects were also tested at 10 a.m. either prior to or nine or more days after the termination of phototherapy ("Off Lights"). The order of treatment conditions was counterbalanced across subjects in each group. Data collection took place between the end of November and the end of March during two consecutive years.

Subjects performed visual and auditory reaction time tasks. The clinical state of all subjects was assessed by raters blind to the treatment condition, who administered the Hamilton rating scale for depression prior to each testing session.

As expected, mean ratings of depression in the patients decreased dramatically following phototherapy ($p < .0001$). There was, however, a wide range of clinical response in the patients, reflected in decreases of 0 to 96% in Hamilton scores after phototherapy.

For patients who improved with treatment, phototherapy was associated with an enhanced P300 to visual but not to auditory stimuli. The P300 did not change in either modality in patients who did not respond clinically to phototherapy. Nor was there any change in the normal controls. Using a strict remission measure, 65% of the patients responded to treatment. The phototherapy-induced increase in visual P300 amplitude was significant ($p < .05$) in the 11 responders, as compared with the 11 normal controls. No change was evident in the auditory modality.

For the patient group as a whole, clinical improvement was highly correlated with the increase in the amplitude of the P300 elicited by visual

stimuli ($r = -.71$, $p < .005$). In contrast, clinical response was uncorrelated with changes in P300 to auditory stimuli ($r = -.18$). Moreover, neither the latency of P300, nor the speed or accuracy of performance, correlated significantly with the clinical ratings.

In patients for whom phototherapy is effective, the effect on P300 is rapid. We showed this in a separate sample of 5 patients and 2 normal controls. ERPs were collected and depression ratings were done at baseline and after 2, 3, and 10 days of phototherapy. In patients who exhibited a favorable response to phototherapy, there was a rapid and progressive increase in P300 amplitude with continued light treatment that was unique to the visual modality. This finding suggests that patients mobilized more visually-guided attention with continued phototherapy.

These changes in P300 mirrored the patients' clinical responses. The within-subject correlations between visual P300 amplitude and the Hamilton score across the four sessions ranged from $-.94$ to $-.30$ ($\bar{X} = -.64$ via the z transformation). In contrast, in the controls, no systematic changes in P300 or Hamilton ratings were apparent in the multiple evaluations of both modalities.

It is evident that phototherapy enhanced visual, but not auditory, P300 in direct proportion to its clinical effect. The amplitude of the visual P300 thereby provides an objective index of the antidepressant effects of light. Our data thus provide evidence against the assertion that phototherapy exerts its action solely by means of a placebo effect.

We have shown that the clinical response to phototherapy in patients with SAD is highly correlated with increased visual attention. Because neither visual nor auditory components earlier than P300 were related to clinical response, phototherapy appears to affect cognitive rather than sensory processing. SAD patients frequently complain of difficulties in concentrating. The awareness of increased visual attention (as reflected by P300) in these patients may lead to lifting of their depressed mood. Alternatively, the mood change may lead the change in attention. Or, both changes could be secondary to modification of some other function.

Since no changes were noted in the auditory P300, the results suggest that phototherapy specifically modifies visual attention in SAD. Because the change in visual P300 amplitude occurs rapidly (within a couple of days of the initiation of treatment), it is a candidate for an effective predictor of those who will benefit from phototherapy. Moreover, P300 may aid in establishing subcategories of affective disorders that are responsive to phototherapy.

2. Schizophrenia

During the past 15 years, a number of studies have shown that the amplitude of the P300 component of the ERP is reduced in schizophrenic patients. The amplitude of P300 has been shown to be a sensitive index of

attention deployment, so that its reduction in schizophrenic patients is consistent with the behavioral findings. However, since the P300 is derived from cerebral electrical activity, it offers the potential to study dynamic brain function. Thus, the P300 component is an attractive tool to investigate putative neurobiological mechanisms underlying the attention deficit in schizophrenia. We extended and broadened the finding of attenuated P300 in schizophrenic patients by evaluating the relative effects of stimulus modality and probability.

To date, we have tested 74 patients (including 30 unmedicated) who met DSM-III criteria for schizophrenic disorder. A group of 30 normal controls, matched on race, sex, age, and education, was also tested. The data showed that, as in previous studies, the P300 was smaller in the schizophrenic patients than the normal controls. However, this difference was significant only for low probability stimuli in the auditory modality and was not found to be significant in the visual modality, suggesting that schizophrenic patients have a greater deficit in auditory than in visual processing. This finding may provide a clue to the underlying pathophysiology and is reminiscent of the relative prevalence of auditory as compared with visual hallucinations in schizophrenic symptomatology.

We sought to determine whether this P300 reduction observed in schizophrenic patients is a reflection of a core deficit, independent of clinical state, or whether it is a reflection of clinical symptomatology. That is, is the reduced P300 a trait as contrasted with a state marker of the disorder? To address this question, 11 schizophrenic patients were tested when they had been stabilized on neuroleptics and again when they had been free of medication for at least 4 weeks. To assess the stability of the measures, 11 matched normal controls were also tested twice, at approximately the same intertest intervals as the patients.

Clinical state was assessed with the Brief Psychiatric Rating Scale. Patients who exhibited the most clinical improvement showed the greatest increase in P300 to visual stimuli ($\bar{r} = -.88$, $p < .01$). In contrast, clinical response was uncorrelated with changes in P300 to auditory stimuli ($\bar{r} = .13$). No change in P300 was apparent in either modality between the first and the second evaluation of the controls.

These data suggest that visual P300 amplitude in schizophrenic patients is inversely correlated with symptom severity. This effect appears to be mediated by the patient's responsiveness to neuroleptic medication. The increase in visual but not auditory P300 amplitude is consistent with the hypothesis that successful neuroleptic treatment enhances a patient's capacity to process visual but not auditory information. Because auditory P300 amplitude was not correlated with clinical state, it remains a candidate for a vulnerability trait marker of schizophrenia. Our data, in fact, suggest that auditory P300 appears to be significantly more sensitive to differences between schizophrenic and normal persons than is visual P300. Moreover, it is clear that mere treatment with neuroleptic medication alone, without symptomatic change, is insufficient to alter visual P300 amplitude. It is

conceivable that the core deficit in schizophrenia is more closely related to relatively invariant impaired auditory information processing and that fluctuations in clinical symptomatic state are reflected in visual processing.

3. Absence Epilepsy

Brain event-related potentials were used to study information processing in the interictal period in a group of 8 patients with absence epilepsy and a matched group of 8 normal controls. ERPs were recorded during performance of visual and auditory versions of the continuous performance test (CPT) of sustained attention.

Differences in reaction time and in omission errors on the CPT between the two groups confirmed that absence patients are greatly impaired in their ability to sustain attention. The behavioral differences seen on the CPT were paralleled in the P300 amplitude data, suggesting that the failure of absence patients to perform efficiently on the CPT is due, at least in part, to inability to mobilize and sustain attentional capacity. This was most pronounced in the auditory versions of the task.

A longer latency N100 in the patients indicated delayed perceptual encoding, although a normal latency P300 component suggested that the higher-level processes of identifying and categorizing a stimulus proceed at a normal rate. The slower responses of absence patients thus appear to be due not to increased stimulus processing time but to prolonged response processing.

4. Eating Disorders

Persons with eating disorders appear to be characterized by altered cognitive processing. In particular, there have been reports that patients with anorexia nervosa may be impaired in automatic but not in controlled processing. We used ERPs to assess these aspects of information processing.

All patients were women who met DSM-III criteria for anorexia nervosa ($n = 24$) or normal weight bulimia ($n = 34$). A group of 34 matched normal controls was also studied. Auditory and visual versions of four reaction time tasks, which varied along the automatic-controlled dimension, were employed to elicit ERPs.

Preliminary analysis of the data indicates separation on the ERP measures between anorexic and control and between anorexic and bulimic subjects. Specifically, anorexic patients showed disturbances in automatic processing, as indexed by a component reflecting an automatic cerebral mismatch process. Altered controlled processing, as measured by P300 amplitude, was also seen in the anorexics; the difference increased with increasing task demands. Bulimics were not distinguishable from controls on the measures. Preliminary findings after long-term weight restoration in 12 anorexic patients indicate reversal in all of the ERP abnormalities except one: The Slow Wave component

following P300 was significantly enhanced in these patients, indicating a relatively permanent disturbance in controlled information processing.

5. Clonidine

A number of investigations have found that malnourished, underweight patients with anorexia nervosa have decreased concentrations of norepinephrine or MHPG in urine, plasma, or cerebrospinal fluid. More recently, several studies have suggested that underweight anorexic patients have increased alpha-2 adrenoceptor activity. Such findings suggest that the functional activity of noradrenergic systems is reduced in these patients. A major CNS noradrenergic pathway originates in the locus ceruleus, which, by virtue of its widespread cortical and subcortical connections, appears to play a role in the maintenance of such functions as attention, sleep, and wakefulness. We have demonstrated that there is an attentional deficit in women with anorexia nervosa that is reflected in changes in the ERP. This study was designed to explore the possible role of locus ceruleus pathophysiology in the attentional disturbance in anorexia nervosa. Clonidine, a relatively specific alpha-2 adrenoceptor agonist, is reported to decrease locus ceruleus activity. This drug was given to healthy, normal women to determine whether it would produce ERP changes similar to those observed in anorexic patients. Such changes would support the hypothesis of a disturbance in locus ceruleus function in this disorder.

Eight healthy young women were administered, on separate days, three doses of clonidine (0.5, 1.0, and 2.0 micrograms/kilogram) and two saline placebos infused intravenously in a counterbalanced order under double-blind conditions. To assess effects on attention, ERPs were recorded during auditory discrimination tasks.

Results indicate that clonidine produced changes in the ERP that resemble some of the alterations observed in anorexic patients, namely, decreases in the amplitude of the P300 component. The data thus support, in part, the hypothesis of altered locus ceruleus function in anorexia nervosa. However, the lack of effect of clonidine on the early negative component suggests that neurochemical systems other than the noradrenergic locus ceruleus system may underlie the reduction in this component in anorexic patients.

6. Dyslexia

We used ERPs to study information processing in a group of 15 adult males with severe developmental dyslexia and 15 matched normal controls. Reduced amplitude P300 has been observed in children with reading disorders. Our study was aimed at assessing whether the P300 reduction observed in dyslexic children would also be found in dyslexic adults. Moreover, we used auditory and visual stimuli, presented in separate reaction time tasks of graded difficulty, to evaluate whether any differences were independent of modality and attentional demands.

No group differences in P300 were seen under relatively undemanding task

conditions. However, the dyslexic group showed a widespread reduction in P300 amplitude in the choice reaction time tasks. A trend toward significance ($p = .07$) was seen in the Group x Modality interaction for both P300 amplitude and latency, indicating a tendency for the group differences to be maximal in the visual modality.

The Abbreviated Parent Conners Scale was used to assess whether subjects had a history of attention deficit disorder (ADD). The dyslexic subjects were assigned to two groups, 7 with ADD and 7 without ADD. Additional analyses based on these subgroups revealed that the dyslexics with ADD accounted for the group differences in P300; the dyslexics without ADD were indistinguishable from controls at all electrode sites. Furthermore, whereas the non-ADD dyslexic group showed the same pattern of hemispheric involvement as controls in both modalities (right left), a trend toward a reversed asymmetry was apparent for the ADD dyslexic group. The results thus suggest that a distinct brain organization may characterize those dyslexic adults who have a history of concomitant attention deficit. Our data may ultimately lead to different strategies of remediation in the two subgroups of dyslexic persons.

Significance to Biomedical Research and the Program of the Institute

Since attentional deficit and cognitive dysfunction are characteristic of many neuropsychiatric disorders, it is important to develop a precise empirical and theoretical account of these symptoms. The scalp-recorded ERP is the only noninvasive technique available for studying the dynamic neural activity associated with cognitive processing in human subjects. The ERP provides information on mental events involved in selective attention, stimulus evaluation and decision making, memory, and learning. The temporal resolution of ERPs can support inferences about brain activity on time scales not possible in studies using tissue assays or radioactivity. Because of the noninvasive character of ERPs, patient state can be monitored often enough to assess the effects of specific clinical or experimental variables. The appropriateness of evaluating ERPs in studies of attention is apparent, as they may provide a dissection of the various components involved and thereby permit more precise identification of the types of information processing deficits responsible for poor performance on attention tasks in a variety of patient groups. It is hoped that the developing battery of ERP and neuropsychological tests applied to patients characterized by attentional deficit and cognitive dysfunction will ultimately provide a neurobiological profile of each disorder and lead to more refined subcategorizations and, eventually, to more efficacious treatments.

Proposed Course

We are currently completing data collection on our studies of patients with eating disorders and schizophrenia. Data collection is complete and analysis is in progress on our studies of seasonal affective disorder, absence epilepsy, dyslexia, and clonidine. We plan to implement new investigations of patients with schizophrenia, affective disorder, and epilepsy.

We plan to extend our studies of affective disorder patients in depth as well as scope. In the seasonal population, we are interested in whether an even more rapid change in P300 may be measurable. If so, this would be of predictive value in evaluating which patients are most likely to benefit from light therapy. It would also be of theoretical value in helping to evaluate the time course of the neurobiological processes underlying the effect of phototherapy. In addition, we plan to study P300 in other types of affective disorder patients to assess the specificity of our findings.

Our work is aimed at illuminating the neurophysiological bases of the attentional and cognitive deficits in affective disorder, schizophrenia, and other psychiatric disorders. Of interest are the relation of ERP variables to diagnosis, diagnostic symptomatology, severity of disorder, degree of formal thought disorder, performance on tests of attention, memory, and intellectual functioning, degree of improvement during treatment, and improvement on specific treatments.

We plan to expand our investigation of the interrelation among ERP components and neuropsychological and neurochemical variables. To increase our understanding of the etiology of schizophrenia and affective disorder, we are planning additional studies to differentiate state versus trait attributes of the disorders. The strategy we intend to use is to compare normal controls with patients when they are actively symptomatic and when they are in remission. We plan to begin testing first-degree relatives of psychiatric patients to determine whether the ERP is a marker of specific disorder. We have implemented selected studies with head-injured cases to test hypotheses derived from various clinical groups concerning the involvement of brain structures in the pathophysiology of psychiatric disorders. Electro-physiological predictors of clinical response to psychopharmacological and other forms of treatment will be sought, as patient availability allows.

Deficits in the regulation of attention have been implicated in several disorders of childhood. Terms used to describe such deficits include hyper- and hypoarousal, distractibility, and filtering failures. We are recording ERPs elicited by stimuli of different modalities and probabilities as part of a comprehensive evaluation designed to differentiate patterns of dysfunction in attentive mechanisms across diagnostic groups. We have begun data collection in a study comparing groups of unmedicated, 6- to 10-year-old children diagnosed as: (1) attention deficit with hyperactivity disorder (ADHD), (2) Tourette's Disorder (TD), or (3) both ADHD and TD. As many as 50 to 60% of children with TD referred to clinics also satisfy the diagnostic criteria for ADHD. Although there is conflicting evidence, some studies have suggested that TD and ADHD may be related genetic disorders. In addition to the clinical groups, a group of children with no evidence of neuropsychiatric disturbance will serve as a normal comparison group. ERP measures will contribute to an understanding of the similarities and differences of attentive functioning across these groups.

We are about to begin a study comparing ADHD children differing in temperamental characteristics. Recent research suggests that children with

attentive and concentration problems are often excessively shy and/or aggressive. This division of ADHD may represent valid subtypes, with varying risks for various maladaptive outcomes. ERP analysis will be used to examine differences in the pattern of attentive difficulties between these subgroups.

Publications

Duncan CC, Perlstein WM, Morihisa JM. The P300 metric in schizophrenia: effects of probability and modality. *Electroencephalogr Clin Neurophysiol* 1987;(Suppl 40):670-4.

Rosenthal NE, Skwerer RG, Sack DA, Duncan CC, Jacobsen FM, Tamarkin L, Wehr TA. Biological effects of morning plus evening bright light treatment of seasonal affective disorder. *Psychopharmacol Bull* 1987;23:364-9.

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Duncan CC. Application of event-related brain potentials to the analysis of interictal attention in absence epilepsy. In: Myslobodsky MS, Mirsky AF, eds. *Elements of petit mal epilepsy*. New York: Peter Lang, 1988, in press.

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Mirsky AF, Duncan CC. Attention impairment in human clinical disorders: schizophrenia and petit mal epilepsy. In: Sheer DE, Pribram K, eds. *Attention: cognition, brain function, and clinical application*. New York: Academic Press, 1988, in press.

Skwerer RG, Jacobsen FM, Duncan CC, Sack DA, Tamarkin L, Wehr TA, Rosenthal NE. The biology of seasonal affective disorder and phototherapy. *J Biol Rhythms*, 1988, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02288-04 LPP
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies on Etiological Factors in Schizophrenia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Seymour S. Kety, M.D. Senior Scientist, NIMH Others: Loring Ingraham, Ph.D. Staff Fellow, LPP, NIMH Paul Wender, M.D. Prof Psychiatry, Univ. of Utah Bjorn Jacobsen, M.D. Assoc Prof Psychiatry, Univ. of Copenhagen Fini Schulsinger, M.D. Prof. Psychiatry, Univ of Copenhagen Dennis Kinney, Ph.D. Asst Prof Psychiatry, Harvard University		
COOPERATING UNITS (if any) Psychological Institute, Copenhagen, Denmark; McLean Hospital, Belmont, Mass.; Harvard University; University of Utah.		
LAB/BRANCH Laboratory of Psychology and Psychopathology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 2.0	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Studies of the occurrence of mental illness in families have been useful in identifying familial forms of the illnesses and in the development of hypotheses regarding the form and strength of <u>genetic</u> and <u>environmental</u> factors in etiology. Where these major variables are separated by the process of <u>adoption</u>, specific <u>etiologic</u> hypotheses can be tested separately and in combination. In our national register of the nearly 1500 Danish adoptees who have reached maturity, classical and borderline <u>schizophrenia</u> occurred at a significantly elevated rate in the biological relatives of chronic schizophrenic adoptees and not in their adoptive relatives, as reported in the previous annual report. This provides compelling evidence for the significant operation of genetic factors in the etiology of this disorder. The increased prevalence does not differ significantly from that in the natural families of schizophrenics, indicating that the well established familial tendency in this disorder is an expression of genetic factors, and providing hitherto lacking justification for the use of family studies to examine the modes of genetic transmission and to search for genetic linkages in large pedigrees of naturally reared schizophrenics.</p> <p>The research this year has focused on the distribution of schizophrenic and all other mental illness in the biological relatives of a large number of adoptees in the Danish National Sample representing several different classes: from adoptees never hospitalized with mental illness and with no present or past evidence of other than trivial mental illness on psychiatric interview, to adoptees diagnosed as classical chronic schizophrenia. Several of the families may be useful for genetic linkage studies and efforts are being made to expand the pedigrees and extend them into additional generations.</p>		

Project Description

Objectives

The objective of this phase of the study of schizophrenic adoptees and their families has been to extend the survey, initially confined to the city and county of Copenhagen, to all of Denmark, evaluating the strength of genetic and family-related environmental influences, to examine the genetic relationships among classical schizophrenia, marginal schizophrenic illnesses, and other mental disorders, and to define more explicitly the traits which may comprise the syndromes of familial and sporadic schizophrenia.

Adoption Study of Schizophrenia

Comprehensive interviews with the biological and adoptive relations of 34 schizophrenic adoptees and non-schizophrenic control adoptees in Copenhagen, rated blindly, had found a significantly higher prevalence of schizophrenia and schizophrenia-related disorder in the biological relatives of schizophrenic adoptees than in controls.

In the 9,000 adoptees outside of Copenhagen, cohorts were identified as having developed schizophrenia, marginal schizophrenia, some non-schizophrenic diagnoses, or being normal. In the course of the past three years, interviews were completed on approximately 90 percent of the relatives of these who are alive and residing in Denmark; these interviews were rated blindly by two experienced judges using global evaluations based on the descriptions of Kraepelin and Bleuler.

During the past year those evaluations were completed and complemented by a review of abstracts of hospital records and reports of incomplete interviews. As was the case in the Copenhagen sample, over 90% of the relatives alive and residing in Denmark, Sweden or Norway participated in a 36 page interview covering a social, psychological and medical history and a complete mental status examination.

The study in Copenhagen found a significant concentration of schizophrenia and schizophrenia-like disorders (chronic, acute, latent and probable schizophrenia, and schizoid personality--the "schizophrenia spectrum of disorders") in the biological relatives of the chronic schizophrenic adoptees. The same finding occurred in the present or Provincial sample representing the rest of Denmark outside of Copenhagen ($p = 0.0004$). Further breakdown of the schizophrenia spectrum in the present sample gave results which confirmed those from the Copenhagen study: "acute schizophrenia" (a diagnosis similar to DSM-III schizophreniform disorder) and "schizoid personality" did not occur significantly more often in the biological index relatives than in those of the controls; if these two categories are excluded from the spectrum, its preponderance in the biological relatives of the chronic schizophrenic (index) adoptees is considerably enhanced as it was in the Copenhagen sample ($p = 0.00003$). Chronic schizophrenia appeared only in the biological index relatives ($p = 0.036$). Probable chronic schizophrenia as well was absent from the relatives of the controls and if the results for

definite and probable schizophrenia are added the results are even more striking ($p = 0.008$).

Absence of a concentration of schizophrenia in the index adoptive relatives does not argue against the importance of environmental factors in the etiology of schizophrenia. In addition to the several hundred environmental variables examined in the interviews, efforts were made to obtain specific information relating to current hypotheses. These results are presently under examination and analysis.

The patterns of mental illness in the biological parents, siblings, and half-siblings of the various groups of adoptees in the Copenhagen and Provincial samples have now been elucidated. For the chronic schizophrenic probands of apparently familial type, a number of interesting configurations appear in their families. There is a tendency for schizophrenic illness to occur unilaterally in either the maternal or paternal relatives. Where schizophrenia is found in a parent, the risk for schizophrenic illness in the half-siblings arising from that parent is close to 50% (11 of 18). Both of these patterns are compatible with dominant monogenic transmission; in fact, the presence of half-siblings, of which there are many in the biological relatives of adoptees, makes such pedigrees potentially high informative. Another advantage of these pedigrees lies in the separation of the proband environment from that of the rest of the family, so that concordance in traits or disorders between the two is likely to be genetic rather than the result of environmental factors.

A deficiency of these pedigrees for linkage studies is that they are limited to only two generations. A search has now been initiated through the population and psychiatric registers for information regarding avuncular relatives and offspring of the probands.

The distribution of mental disorders in the biological relatives of the probands with various diagnoses appears to support a few hypotheses concerning the genetic relationships between syndromes or their specificity and overlaps. The 136 adoptees who served as probands in the Copenhagen and Provincial Studies can be broken down into the following diagnostic groups, the diagnoses having been made without any knowledge regarding their biological or adoptive families: 48 control probands never hospitalized for mental illness and free of present or past indications of significant mental disorder on psychiatric interview, and 49 with classical chronic schizophrenia (of whom 28 were found to have schizophrenia or borderline schizophrenia in their biological families and 21 were not). In addition, there were 23 probands with marginal types of schizophrenic illness (acute schizophreniform, schizoaffective, or schizotypal), 9 with major affective disorder, 4 with hysterical psychosis, and 3 with schizoid personality.

In the biological relatives of the probands listed above, the distribution of mental illness was as follows:

1. Among the 290 biological relatives of the 49 classical chronic schizophrenic adoptees, there were 15 diagnoses of chronic schizophrenia (5.17%). No instances of chronic schizophrenia occurred in the biological

relatives of the other probands except for the 15 latent schizophrenic probands where 2 such diagnoses occurred in their 73 biological relatives (2.74%). If there is indeed a familial form of schizophrenia, the 28 such probands had the 15 instances of chronic schizophrenia in their 180 biological relatives (8.33%). In any case it appears that chronic schizophrenia "breeds true," appearing almost exclusively in the biological relatives of such probands.

2. Schizotypal disorder was found at a relatively high prevalence in the biological relatives of the chronic schizophrenia probands (11.03%). This would support the conclusion that this marginal disorder was genetically related to schizophrenia. However the diagnosis or the relationship to schizophrenia is not specific since there is a high frequency of schizotypal disorder diagnoses among the biological relatives of manic depressive (17.07%), hysteric and schizoid (8.8%) probands, but not among those of the controls (1.65%).

3. There is an unexpectedly high prevalence of major affective disorder (7.27%) in the biological relatives of the "non-familial" schizophrenic probands, but not of the "familial schizophrenic" adoptees, suggesting that misdiagnosis of manic-depressive as schizophrenic illness is more likely to occur where there is no family history of schizophrenia.

Significance to Biomedical Research and to the Program of the Institute

These findings confirm and extend the results obtained previously indicating a strong and quite specific genetic influence in the transmission of classical schizophrenia. They also support a genetic relationship between a milder syndrome--latent schizophrenia of DSM-II or schizotypal personality of DSM-III--and classical schizophrenia, although this milder syndrome is not specific to the relatives of chronic schizophrenic subjects alone. These observations also indicate that the well known tendency of schizophrenia to be concentrated in families is the result of genetic rather than family-associated environmental factors and validate the usefulness of family studies of nonadopted schizophrenics for the examination of genetic influences. A major implication of the operation of genetic factors in etiology is the recognition of the importance of biological, and especially, biochemical factors in this disorder, since the genes can only express themselves through biochemical processes. Another important result of the more conclusive genetic evidence derived from adoption studies in schizophrenia, already realized, is the scientifically based alternative to the widely promulgated hypothesis of the "schizophrenogenic" parent and deleterious rearing practices as major etiological factors in schizophrenia.

Proposed Course

Environmental variables pertaining to socioeconomic class, history of infections and dietary habits, rearing practices, personality of rearing parents, language patterns, communication deviance, expressed emotion, as well as evaluation of cognitive function such as measures of thought disorder, a psychobiological test of smooth pursuit eye movements and one biochemical

measure were included in the new data gathered with the interviews in the Provincial sample. These are being analyzed and correlated with the clinical and demographic data at hand to examine existing hypotheses and possibly to generate some new ones regarding the environmental influences operating in schizophrenia.

The interview data, particularly the large number of items in the mental status examination, will be analyzed to specify more accurately the symptoms and manifestations found among the biological relatives of the chronic schizophrenic probands, with the aim of identifying traits genetically associated with schizophrenia and developing more specific characteristics of syndromes genetically related to schizophrenia in the biological relatives of chronic schizophrenic adoptees.

A third sample of schizophrenic adoptees has been identified in Denmark, representing adoptees with onset of illness and hospitalization after the previous search through the adoption and psychiatric registers. The biological and adoptive relatives of this sample, with suitable controls, will be examined for mental illness on the basis of hospitalization alone, deferring perhaps indefinitely the expense of the exhaustive psychiatric interviews that have characterized the two previous samples. There is reason to believe that in Denmark the number of chronic schizophrenic individuals who never reach a mental hospital is very small.

Publications

Kety SS, Matthysse S. Genetic and biochemical aspects of schizophrenia. In: Nicholi A, ed. Harvard Modern Guide to Psychiatry, Cambridge, MA, Harvard University Press, 1988.

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Kety SS. Schizophrenic illness in the families of schizophrenic adoptees: findings from the Danish national sample. Schizophr Bull 1988;14:217-222.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02295-03 LPP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Factors in Response to Alcohol

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Connie C. Duncan, Ph.D.	Chief, Unit on Psychophysiology	LPP/NIMH
Co-PI:	Frances H. Gabbay, Ph.D.	Guest Researcher	LPP/NIMH
Others:	Allan F. Mirsky, Ph.D.	Chief	LPP/NIMH
	T. Peter Bridge, M.D.	Deputy AIDS Coordinator	ADAMHA

COOPERATING UNITS (if any)

Department of Mental Hygiene, School of Hygiene and Public Health, Johns Hopkins University

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to assess the relative contributions of genetic and environmental factors to alcohol drinking and response to alcohol challenge. The project is composed of three studies. In the first, male monozygotic (MZ) and dizygotic (DZ) twin pairs complete a 28-day record of their alcohol intake. These data will permit estimation of the relative contributions of genetic and environmental factors to frequency, amount, and pattern of alcohol consumption. In addition, self-reports of contact between cotwins will permit estimation of the importance of shared environment on cotwin similarities in alcohol drinking.

In the second study, male MZ and DZ twin pairs receive, in three separate testing sessions, a placebo and two doses of alcohol (0.40 and 0.80 g/kg of 95% ethyl alcohol). The protocol consists of electrophysiological measures (e.g., brainstem auditory evoked responses, spectrally analyzed EEG, and visual and auditory event-related potentials), a behavioral measure of attention, self-reports of affect, and a measure of standing stability. The use of placebo and multiple doses of alcohol will permit conclusions about the effects of alcohol on information processing, response production, mood, and motor activity. The twin design will provide information on the relative contributions of genetic and environmental factors to variability in these measures in the drug-free state and following response to alcohol challenge.

In a third study, nontwin males are tested in two separate sessions using a protocol identical to that employed in the twin study. This study will permit estimation of the retest stability of the alcohol effects. This information will be critical in the dose-response analysis and in the genetic analyses of the twin data.

A. Objectives

The primary and most significant objective of this project is to estimate the relative contributions of genetic and environmental factors to variability in alcohol drinking and response to alcohol challenge. In addition, the project will test the effects of alcohol on information processing and response production, on attention and mood, and on motor activity, and examine the stability of alcohol drinking, electrophysiological activity, and response to alcohol across time within individuals.

B. Methods Employed

Survey Study. In the first study, male twin pairs (MZ=142 and DZ=73) have completed or are currently keeping a 28-day record of their alcohol intake. Diary entries are converted into grams of absolute alcohol consumed per day. From these values, seven variables describing twins' alcohol use are computed (e.g., grams of alcohol consumed per weekend day, number of days drinking, maximum amount consumed in a day). Intraclass correlations will be used to assess intrapair similarity, and a comparison of MZ and DZ correlations will provide information on additive vs. nonadditive genetic effects, random vs. assortative mating, family environmental effects, and gene-environment covariance. A self-report questionnaire bearing on the relationship between cotwins is also being administered to assess the extent to which cotwins interact. These questionnaire data will be employed to determine to what extent this component of shared environment accounts for similarity in the frequency, amount, and pattern of alcohol drinking.

Twin Study. In the second study, male twin pairs (ages 21-35) are being studied in the laboratory as they become available. All subjects are moderate drinkers of alcohol and are in good health as determined by a physical examination. Twins who meet DSM-III criteria for Alcohol Abuse or Dependence Disorder, or for other psychiatric disorders, and those with family histories of psychiatric illness are excluded from testing. Over the course of three experimental sessions, twins ingest a placebo, 0.40 g/kg, and 0.80 g/kg 95% ethyl alcohol. The test protocol includes electrophysiological measures (brainstem auditory evoked responses (BAERs), spectrally analyzed EEG, and visual and auditory event-related potentials (ERPs)); a behavioral measure of attention (the Continuous Performance Test (CPT)); self-reports of affect; and a measure of standing stability. Baseline recordings are made, and, after the beverage, additional recordings are made. ERP measurements are taken once again and the CPT is readministered. Resting EEG, BAERs, standing stability, and the affective measure are repeated three additional times. Breath samples are taken every 10 minutes to provide estimates of blood alcohol levels. Intraclass correlations will be computed to assess intrapair similarity in these measures in the drug-free state and following alcohol challenge. Repeated measures analyses of variance will be used to test the effects of alcohol on these measures. Pearson correlations will be used to assess the relationships among the various measures (e.g., EEG and affect) and, using the baseline recordings, to estimate the stability of these measures across time within subjects.

Reliability Study. Nontwin males are administered 0.80 g/kg 95% ethyl alcohol, in two separate sessions (with one to four weeks intervening). All other screening and recording procedures are identical to those used in the twin study. A Pearson correlation coefficient will be used to assess the retest reliability of the alcohol effects on each of the measures across the two test sessions.

C. Major Findings

These studies are in progress, but preliminary statements may be made on the basis of examination of the data collected to date.

Survey Study. Preliminary results from the first study indicate that MZ twins are strikingly similar in the amount of alcohol consumed and in their patterns of consumption. Intraclass correlations for the variables derived from the 28-day diaries range from .77 to .91. In contrast, estimates of within-pair similarity for DZ twins indicate that they are, on the average, far less similar than MZ pairs in their patterns of alcohol consumption. This suggests that, for males, genetic factors play a role in moderate alcohol consumption patterns, a finding that is consistent with previous research. The pattern of MZ-DZ correlations (e.g., DZ correlations that equal less than half the corresponding MZ correlation) suggests that the genetic contribution is not strictly additive.

Twin Study. Testing of twins is underway, but no twin pairs have completed the protocol. Data from non-twin pilot subjects, from individual twins, and from nontwin retest subjects permit the following preliminary results (based on visual inspection of the data, not on statistical analysis) to be reported:

1. There appears to be a detectable alcohol effect on the measures we are using. Alcohol caused a slight delay of peaks I through V of the BAER, indicating that transmission time to the brainstem and within the brainstem is slowed by alcohol. Further, alcohol appears to cause a decrease in P300 amplitude of the event-related potential and an increase in its latency, suggesting that alcohol also slows cognitive processing. Spectral analyses of the resting EEG suggests that the EEG is characterized by increased power in the lower frequency range (i.e., theta band) following alcohol ingestion, consistent with the known soporific effects of the drug. Recently collected behavioral data are consistent with these electrophysiological findings. These data suggest that performance on the CPT, a vigilance task, as indexed by reaction time and by number of errors, deteriorates following alcohol ingestion. Conclusions about the affective change and its relationship to the EEG frequency changes await further analyses.

2. Of great importance is the finding that, while these alcohol effects appear in the averaged data and in some individual subjects, the effects are not equally strong or in the same direction for all subjects. We reported previously that one subject showed a decrease in the latency of BAER peaks and that the dampening effect of alcohol on the P300 component of the

event-related potential was not consistent across subjects. Recently collected data show that individual differences characterize the behavioral data as well: although alcohol caused an increase in the mean reaction time, in errors of omission and commission, there were subjects who did not show these performance decrements following alcohol. These findings are consistent with previous findings by the Co-PI, as well as other investigators; but because of statistically significant effects in grouped data, individual differences typically have not been emphasized in research reports.

3. The electrophysiological measures appear to be very stable in the nondrug condition within subjects across time. This retest stability has implications for the analysis of alcohol effects and for the estimation of heritability. Within-subject variability increases error variance and decreases the chances of detecting alcohol effects, while retest reliability puts an upper limit on heritability (i.e., cotwin similarity will be limited by the extent to which the trait is stable within a single subject). Thus, their retest stability reinforces the decision to include these measures in this study and suggests they will prove useful in future pharmacogenetic research.

In the past year, we have added a third study to this project, one designed to test specifically the retest stability of the alcohol effect. Early results from this study suggest the measures are not only stable in the baseline condition, but that variability in these indices of alcohol effects is stable over time as well.

4. Time course effects observed in the early data from this study point to important methodological issues as well as provide potentially important information on the mechanism of drug action. Recordings are made once before and one to three times after alcohol ingestion, and there are differences across subjects and across variables in the course of effects. In some cases, depressant-like effects occurred immediately; while in others, effects were not observed until the second test. As has been reported previously, stimulant-like effects were observed in some cases during the ascending blood alcohol curve, with depressant effects occurring later. Failure to repeat assessment over time would result in incorrect conclusions about the effects, or lack of effects, of alcohol.

D. Significance to Biomedical Research and the Program of the Institute

The significance of the first study lies in its power to estimate the relative contributions of genetics and environment to alcohol drinking in a sample of nonalcoholic males. To date, no such studies based on samples drawn from within the U.S. have been published. Moreover, the use of a diary format to assess alcohol intake will increase the confidence in the validity of the findings. Finally, the quantification of the extent of social interaction between cotwins and the use of that estimate to account for within-pair similarity in alcohol drinking will provide information on the nature (rather than just the size) of the environmental contributions to variability in alcohol drinking.

The significance of the second study derives from its use of powerful electrophysiological measures of brain activity within the context of a pharmacogenetic design. This project will shed light on information processing and response production, on disruptions in these processes resulting from alcohol ingestion, and on the heritability of these aspects of brain functioning. Moreover, because response to alcohol may play a role in the development of alcohol abuse and dependence disorders, the finding of individual differences in response to alcohol will be of great importance in the study of alcoholism. The stability of these differences will be established in the reliability study, and information on their heritability will be derived from the twin study. Thus, these measures may be more effectively employed in studies of individuals at risk for the alcohol-related disorders to determine whether they are associated with differences in vulnerability to alcoholism.

E. Proposed Course

Survey Study. A sufficient number of twins will have completed these diaries to permit the computation of heritability of aspects of alcohol consumption. As twin pairs become available as the result of additional recruitment efforts, we will add to the sample size to permit the application of multivariate genetic analytic strategies to these data.

Twin and Retest Studies. Collection of laboratory data on the twin sample will continue to proceed as twin availability allows. Over 40 pairs of twins have been recruited through advertising in the Washington, D.C. Metropolitan area, and these twin pairs are currently being screened for participation in the laboratory study. In addition, search efforts are being extended (e.g., to include all post-secondary institutions in the greater Washington, D. C. Metropolitan area). Data bearing on the retest stability of the alcohol effect will be collected concurrently from a sample nontwins. Data analysis and preparation of the results for publication and presentation will be conducted as the data are collected.

Caffeine Study. A fourth study is being planned in which a similar protocol will be employed to study individual differences in response to a stimulant drug. In five sessions, a placebo, two doses of caffeine (4 and 8 mg/kg), and two doses of alcohol (.4 and .8 g/kg) will be administered to male volunteers ages 21-35. Subjects will be moderate alcohol drinkers and caffeine users. This design will permit 1) dose-response analyses of caffeine effects on electrophysiological indices of information processing, and 2) analyses of the relationships among variability in stimulant and depressant drug response.

F. Publications

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02404-02 LPP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychophysiological Investigations of Preattentive and Attentional Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Bruno J. Anthony, Ph.D.

Senior Staff Fellow

LPP, NIMH

COOPERATING UNITS (if any)

Department of Mental Hygiene, Johns Hopkins School of Hygiene and Public Health; Baltimore City Public Schools

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to examine dysfunction of preattentive and attentive regulatory mechanisms in children and infants who exhibit disturbance in the modulation of cognitive, affective, and social processes. Actions of these regulatory mechanisms are assessed through examination of steady-state autonomic and central nervous system activity and of the response of these systems to environmental events. Measurement of the blink reflex, heart rate, respiration, and task performance during rest and in response to simple two-stimulus paradigms permit assessment of the integration of different neural systems (sensory, motor and autonomic), preattentive inhibitory and excitatory effects on sensory processing, and different components of attention including intensity, maintenance, breadth (focus or selectivity), and resistance to distraction. In the first year of this project, laboratory and computer facilities have been developed for stimulus presentation as well as data acquisition and analysis. Groups of normal infants and children were tested to examine developmental patterns in regulation. A study comparing differences in regulatory function in groups of 6- to 11-year-old children with behavioral patterns of attentive dysfunction is underway. These groups include children with Attention-deficit Hyperactivity Disorder (ADHD), with Tourette's Disorder (TD), and with both ADHD and TD.

Project DescriptionA. Other Personnel

Allan F. Mirsky, Ph.D.	Chief	LPP, NIMH
Connie C. Duncan, Ph.D.	Senior Staff Fellow	LPP, NIMH
Frances H. Gabbay, Ph.D.	Guest Researcher	LPP, NIMH
	Research Associate	Johns Hopkins
	Dept. of Mental Hygiene	University
Sheppard G. Kellam, M.D.	Chairman, Department of	Johns Hopkins
	Dept. of Mental Hygiene	University
William W. Eaton, Ph.D.	Associate Professor,	Johns Hopkins
	Dept. of Mental Hygiene	University
Mary Beth Ahearn	Graduate Assistant,	Johns Hopkins
	Dept. of Mental Hygiene	University

B. Objectives

The project is designed to examine neurobiological indices of dysregulation, their variation across neuropsychiatric disorders in adults and children and across temperamental styles in infancy, and their predictive validity for later development of behavioral and neuropsychiatric difficulties. Employing surface recordings of autonomic and central nervous system activity, these studies will measure attentive processes and preattentive, automatic processes that underlie adequate attention. The project is principally concerned with, but not confined to, studies of infants and children. Patterns of deficits will be examined in children and adults with specific neuropsychiatric disorders (Pervasive Developmental Disorder, Attention Deficit Disorder, Stereotyped Movement Disorders and Anxiety Disorders) or with neurological impairments (seizure disorders, brain damage) as well as those individuals at risk because of familial psychopathology or symptomology related to specific disorders. The project is also aimed at examining psychobiologic indices of regulation in infancy and their relation to measures of temperament and the role of genetic and environmental factors in accounting for variance in these measures. In addition, groups of infants and children will be followed longitudinally to examine the developmental course of attentive and preattentive deficits, and the relationship between these deficits and the development of neuropsychiatric problems.

C. Methods Employed

In selecting measures, we considered the regulation of steady state neural activity as well as the regulation of reactivity to environmental events through preattentive as well as attentive mechanisms. The psychophysiological methods employed in the present work--startle blink and autonomic responses--allow for a direct and powerful way to assess regulatory activity. Also, it should be noted that attentive and preattentive functions in many patients enrolled in this project are also assessed neuropsychologically (see project description Z01-MH-00508) and through event-related potential testing

(see project description Z01-MH-00509). Because of their sensitivity, their obligatory nature and their ease of measurement, these psychophysiological measures are ideal tools for assessing and comparing subjects of all ages and capabilities.

Regulation of resting autonomic nervous system activity is assessed through examination of the balance of sympathetic and parasympathetic subdivisions. This balance is indexed through analysis of a specific type of HR variability, respiratory sinus arrhythmia (RSA). This measure reflects the rhythmic increases and decreases in heart rate partially caused by the influence of the brainstem respiratory centers on the vagal efferents to the heart. Substantial individual variation in the extent of "coupling" of HR and respiration appears to reflect variation in the generalized integration of many behaviors and physiological systems which depend on transmission through the brainstem. Resting central nervous system activity is assessed, in part, through examination of spontaneous blink rate which appears to be influenced by central dopamine activity.

The psychophysiological battery also assesses inhibitory and excitatory regulation of autonomic and central nervous system responses to environmental events. A major thrust of the proposed work is to study dysfunction in preattentive regulation. Such a distortion has been suggested as contributory to a variety of neuropsychiatric disorders; but it has been difficult to assess preattentive processes with traditional, information processing methods. However, changes in startle blink and cardiac reflexes brought about by prior stimulation have been shown to reflect the adequacy of important inhibitory (filtering), excitatory (arousal), and sensory integrative functions operating without attentional or conscious control.

The same psychophysiological measures are used to examine control of responsivity accomplished through higher-level attentive mechanisms. Heart rate deceleration is used to establish the presence and intensity of phasic and sustained attention, which is mobilized by subjects as they perform sensory discrimination and/or reaction time tasks. The selective component of attention and the ability to resist distraction is assessed by examining the effect of attentive focus on the startle blink. Blink magnitude is enhanced and latency shortened when attention is directed to stimuli in the same modality as the eliciting stimulus; conversely, when attention is directed away from the modality of the reflex probe, blink magnitude is diminished and latency, while still reduced, is shortened less than when probe and eliciting stimuli are in the same modality.

Behavioral aspects of regulation are obtained from semi-structured diagnostic interviews with parent and child using the Child Assessment Schedule (CAS), a parent interview on adaptive behavior (Vineland Adaptive Behavior Scales), parent questionnaires (Achenbach Child Behavior Checklist, Connor's Parent Questionnaire), and teacher-completed checklists (Connor's Teacher Questionnaire, Teacher Observation of Classroom Adaptation).

D. Major Findings

In the first year of this protocol, we have revised and developed computer programs for the delivery and timing of acoustic and visual stimulation, data collection, and data analysis. In addition we have purchased, installed and tested additional equipment for the measurement of autonomic and skeletal muscle responses. In order to assess the ability of the blink and heart rate measures to assess preattentive function in children, we completed a study of 13 normal volunteers aged 6-10 years. The results showed that an inhibitory or gating mechanism designed to filter input at a preattentive level develops slowly and unevenly, continuing to show maturational changes up to age 10. In contrast, the low-level activation process which enhances input appeared fully mature. We also piloted portions of the psychophysiological battery on 7 four- to six-month-old subjects in preparation for a twin study of regulation in infancy described in section F (Proposed Course). This work indicated the feasibility of the battery for use with very young subjects and confirmed our previous findings of a deficient gating function but a mature arousal process in infancy.

We have recently begun a study comparing preattentive and attentive processes in a sample of normal children with groups of children exhibiting different behavioral patterns of attentive dysfunction. Such behaviors occur in a wide variety of neuropsychiatric disorders. Children who are being called ADHD may be inattentive for reasons quite separate from the presumed pathophysiology thought to underlie ADHD. To begin to address the similarities and differences in regulatory function across developmental disorders, we chose to study groups of children diagnosed with ADHD, with Tourette's Disorder (TD), and with both ADHD and TD. As many as 50 to 60 percent of children with TD referred to clinics also satisfy the diagnostic criteria for ADHD. Although there is conflicting evidence, some studies suggest that TD and ADD may be due to the same underlying genetic disorder of regulation. In addition, because of growing evidence that ADHD children who exhibit aggressive/oppositional behavior may represent a distinct subtype, we are comparing those ADHD children who exhibit such symptoms with those who display anxious and inhibited behavior. ADHD seems to be a disorder of modulation that may pervade all aspects of functioning. Differences in the action of preattentive and attentive regulation may vary with affective and social characteristics as well as cognitive abilities.

So far, we have tested 16 children, 13 with ADHD, 2 with ADHD and TD, and 1 with TD. Children in this study participate in a comprehensive, three-day evaluation, which includes neuropsychological and event-related potential testing as well the procedures from the present protocol. Preliminary analyses of the blink and heart rate modulation data indicate that, compared to the group of normal children, the ADHD group, as a whole show adequate preattentive filtering of input but an overresponsive activation system. We plan to continue to test ADHD and TD children, as well as children without an Axis I or II DSM-III-R diagnosis, until we obtain 20 children per group.

E. Significance to Biomedical Research and to the Program of the Institute

The adequate assessment of attentive dysfunction and underlying preattentive dysfunction is critical for the understanding of the neuropsychiatric disorders of childhood and the prevention of later-occurring psychopathology and substance abuse. Many children who do poorly in school and/or exhibit learning difficulties or significant neuropsychiatric disturbances appear to have attentional deficits. In addition, the severity of learning problems increases as the child grows older. One-half of the referrals to the nation's mental health clinics are for attention-related problems. Also, it is becoming increasingly clear that attention disorders can no longer be considered only as problems of childhood. Over half of adolescents with Attention Deficit Disorder are referred to the courts for theft and truancy and one quarter are later diagnosed as possessing Anti-social Personality Disorder. Moreover, attention problems may represent a factor which is either central to, or increases the vulnerability of those children already at genetic risk for serious psychopathology such as schizophrenia.

The increasing focus on disturbed attention as a possible underlying pathological process has generated its own set of difficulties. Attention is a broad-based, complex phenomenon that is more difficult to operationalize and to measure than activity or impulsivity. This work contributes to an understanding of the neurobiologic dysfunction underlying attention disorders, to the separation of more global "attentiveness" into different components, and to the role of regulatory problems in the development of neuropsychiatric problems from infancy through adulthood. As the potential for early intervention increases, it becomes more important to evaluate children as early as possible with an eye to preventative and therapeutic approaches.

F. Proposed Course

We are beginning a study of steady-state regulation of internal systems and regulation of response to external stimulation in infants. Variation in these psychophysiological assessments will be related to dimensions of temperament. Despite a growing literature surrounding the psychobiology of adult psychopathology and personality dimensions, there has been comparatively little research investigating the biological bases of infant temperament and its relation to the development of behavioral problems in childhood. Specifically, the aims of our study are: 1) to determine the stability of both behavioral assessments of temperament and the psychophysiological measures of regulation in six-month-old infants; 2) to examine relationships among the behavioral assessments of temperament and the psychophysiological measurements at six months and at three years; 3) to provide data which bear on the contributions of genetic and environmental factors to temperament differences and to variability in the psychophysiological measures of regulation at six months and three years; and 4) to examine the relationship between the six-month and three-year assessments. To accomplish these goals, 56 same-sex twin pairs (MZ male=14; MZ female=14; DZ male=14; DZ female=14 pairs) will be tested at six months and again at three years. At age six months, the pre-locomotor version of the Laboratory-Based Assessment of Temperament

(LAB-TAB) as well as portions of the psychophysiological battery will be administered. The battery will assess tonic regulation of autonomic and central nervous system activity, as well as phasic mechanisms of responsivity such as preattentive activation and filtering processes, habituation, orienting, and selective attention.

We also plan a longitudinal examination of children attending second and third grade in the Baltimore City Public Schools who will be selected on the basis of their performance on a battery of neuropsychological tests. Analysis of this battery, administered to a representative sample of 435 children, has revealed distinguishable components of attentive functioning (see project summary Z01-MH-0508). In the future, a group of children deemed at highest risk from this battery and a representative sample of the population who do not appear at risk will be brought to the LPP to participate in this project. The goal of this study is to examine and describe neurobiological indices of attention drawn from a unselected population, and of the processes that underlie attention and, further, to examine their relation to behavioral and neuropsychological indices. Moreover, we hope to follow these children longitudinally to examine the stability of these psychophysiological indices and their usefulness as predictors of later maladaptive behavior.

G. Publications

Anthony BJ, Graham FK, Balaban MT. Sensory filtering by extrinsic and intrinsic mechanisms in development: reflex investigations. In: Rovee-Collier C, Lipsett L, eds. Advances in infancy research, in press.

Balaban MT, Anthony BJ, Graham FK. Prestimulation effects on blink and cardiac reflexes of 15-month human infants. Dev Psychobiol;21, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00672-23 LSES

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Social Psychological Correlates of Occupational Position

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. Schooler, Acting Chief, Laboratory of Socio-environmental Studies, NIMH

OTHER: C. Schoenbach
M. KohnSocial Science Analyst
Guest ResearcherLSES NIMH
LSES NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

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TOTAL MAN-YEARS:

3.25

PROFESSIONAL:

.50

OTHER:

2.75

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☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The central aim of this project is to examine how the psychological effects of the different life circumstances faced at different stages of their life course by people in various positions in the social hierarchy help explain the differences found in their psychological functioning. The most important accomplishment this year has been the completion of an examination of how supportive and controlling parental behavior may affect aspects of children's psychological functioning. The results generally support the hypotheses, derived from the Laboratory's research program, that controlling parental behavior decreases children's self-directedness and intellectual flexibility and increases their distress, while supportive parental behavior has opposite effects.

Project Description:

The central aim of this project is to examine how the psychological effects of the different life circumstances faced at different stages of their life course by people in various positions in the social hierarchy help explain the differences found in their psychological functioning. It began in 1964 with structured interviews with a sample of 3100 men, representative of all men employed in civilian occupations throughout the United States. In 1974 follow-up interviews were conducted with a randomly selected one fourth of the original respondents, as well as with their wives and with one of their children. Subsequently, the men's and women's surveys were replicated in Poland, and the men's, women's and children's surveys replicated in Japan.

The central findings have been that self-directed work leads to intellectual flexibility and to a self-directed orientation to self and society and that oppressive working conditions lead to distress. These findings have been found to hold true for a variety of work settings (paid employment, school work, women's housework), at different stages in the life span, and in both Poland and Japan.

The most important accomplishment this year has been the completion of an examination of how some types of parental behavior may affect aspects of children's psychological functioning. The parent's behaviors examined are control and support; the aspects of children's functioning examined are self-directed values and orientations, intellectual flexibility and distress. The latent variable linear structure equation analyses that were used permitted the purging of measurement error from the parents' and children's reports of parental behavior as well as from the measures of the various psychological functions. The analyses controlled the effects, not only of relevant background variables, but also of the parents' psychological functioning. This latter control insures that the relationships found between aspects of children's functioning and their parents' behaviors are actually due to the parents' behaviors and are not artifacts of direct genetic transmission or of some environmental factor linked to both the psychological function and the parental behavior in question. Although reciprocal effects models could not be successfully estimated and despite the various anomalies and sex differences found, the results generally support the hypotheses. These hypotheses, derived from the Laboratory's research program and from the relevant literature, are that controlling parental behavior decreases children's self-directedness and intellectual flexibility and increases their distress, while supportive parental behavior has opposite effects. Most of the significant exceptions to this pattern seem to occur when parents exhibit non-stereotypic sex-role behavior that appears to have positive effects on their children.

Besides finishing their analyses of the effects of parents' behavior on American children, Schooler and Schoenbach have also continued their work

on interview data of Japanese families. These interviews, which were gathered in collaboration with Japanese investigators in Tokyo and Osaka, are essentially replications of the American family interviews and it is anticipated that they will be subjected to a similar course of analysis as were the data from the American families.

Further analyses of the American family data are also continuing. Kohn in collaboration with Schoenbach and Schooler has begun an extensive examination of the social structural determinants of the transmission of psychological functioning across generations. Schoenbach and Schooler are also continuing the examination of the social structural antecedents of children's social behavior. Schooler in addition has written several papers showing how both the substantive and methodological outcomes of this program of research are relevant to a whole gamut of disciplines and how these outcomes can help in the integration and understanding of a wide range of phenomena.

Significance of the Research:

Because the characteristics of both more and less complex levels of phenomena affect intermediate ones, to understand human psychological functioning we have to have a basic knowledge of both the human central nervous system and the human social system. Recognizing the importance of the social level of phenomena in determining human behavior means that the study of the human social system cannot be totally neglected without seriously imperiling the search for the root causes of psychological dysfunction. Although the examination of social level phenomena thus represents a legitimate basic research goal, this project has focused on those aspects of the social environment which we have reason to believe affect the individual's cognitive functioning, interrelationships with others and ability to cope with life's stresses.

These three areas, besides being essential for our basic understanding of human nature, are clearly implicated in mental illness. Schizophrenia, for example, is characterized by cognitive dysfunction, social withdrawal and an inability to cope with social and other forms of stress. Research carried out by the Laboratory indicates that people at lower stratification levels have less effective cognitive and other psychological mechanisms for coping with stress and uncertainty and more rigid and conformist orientations towards others. Thus, oppressive job conditions, poor cognitive and other forms of coping mechanisms, and the nature of the individual's orientation to others are linked to social status in ways that suggest that they may play a part in the etiology of psychological dysfunction. In the study of mental illness basic socio-environmental research must accompany basic research on the molecular and neurological level.

Beyond NIMH's concern with mental disorder is the Institute's mandate to study the conditions that facilitate and those that interfere with effective psychological functioning. This research demonstrates that

social structurally determined environmental conditions have appreciable effects on cognitive performance, self conceptions and orientations to the outside world and thus are directly linked to the mental health and effective psychological functioning of individual.

Proposed Course of Further Research:

Data have been collected on the psychological effects of occupational and other social structural conditions on a sample of Japanese fathers, mothers and children that parallels the data we have collected in the United States. The analyses of the data on the Japanese men have already been completed and reported. We are now analyzing the women's and children's data to investigate the interaction of culture and social structure on the individual's intellectual and interpersonal functioning. This Japanese data will also be used to examine the nature of the intrafamilial transmission of psychological functioning, and of orientations to self and society. The results of these analyses will serve as a basis of comparison with those of similar, presently ongoing analyses of our American data. Such comparisons will permit us to assess the cross-cultural generalizability of our results. They will also help us answer basic questions about the way in which culture interacts with social structure and parental influence to affect the psychological functioning of the individual.

Publications: .

Roberts BR. A confirmatory factor-analysis model of alienation, Soc Psychol Q 1987;50:366-51.

Schaie KW, Schooler, C. eds. Social structure and aging: psychological processes, Hillsdale: Erlbaum, 1988.

Schooler C. Levels and proof in cross disciplinary research. In: Kertzer DI, Meyer J, Schaie KW, eds. Comparative perspectives in age structuring in modern societies. Hillsdale: Earlbaum, 1988.

Schooler C, Naoi A. The psychological effects of traditional and of economically peripheral job settings in Japan, Am J Sociol 1988;94.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00679-08 LSES

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Equation Models in the Analysis of Data with Measurement Error

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ronald J. Schoenberg, Research Sociologist LSES NIMH

OTHER: C. Schooler, Acting Chief LSES NIMH

COOPERATING UNITS (if any)

None

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Laboratory of Socio-environmental Studies

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TOTAL MAN-YEARS:

1.00

PROFESSIONAL:

1.00

OTHER:

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- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The purpose of this work is to further develop the methods and techniques for the specification and estimation of the parameters of structural equation models of survey data that contain random and nonrandom measurement error. Included in this are methods for the identification of the models, estimation of the means of unobserved variables, the determination of model condition, and the treatment of polytomous variables.

PROJECT DESCRIPTION:

During the year two different statistical methods have been extended and adapted, in terms of both programming and theory, for use in the study of schizophrenia. First, the latent class model, a method for the analysis of categorical data, has been applied to two community based data sets containing items measuring symptoms usually regarded to be indicative of schizophrenia ("Do you hear voices", etc.). In this particular application, however, the latent class model has been modified to incorporate techniques of causal modeling that were developed in this laboratory for previous research. The items are hypothesized to be "indicators", or observed measures, of latent properties of the individual that cannot be observed directly.

In an initial model eleven indicators, or observed symptoms, were hypothesized to be measures of a single latent cause which will be labeled "schizophrenia". This model failed to fit, lending evidence to the claim that the set of symptoms cannot be accounted for unidimensionally. Further exploratory work has revealed that at least three dimensions are required to account for the symptoms.

The real contribution of this analysis, though, is not the determination of the dimensionality of the underlying construct, but rather it is in the estimation of the latent table relating the latent dimensions to each other. In the usual latent class analysis the observed items are used to classify cases on latent constructs or dimensions but no attempt was ever made to relate these latent dimensions to each other. With the modification of the latent class method used here a latent table of the dimensions is estimated permitting the analysis of the relationships of the latent dimensions. Traditional latent class analysis more resembles cluster analysis, but the extension of this method is more like confirmatory factor analysis which is a more powerful model allowing the specification of structural relationships among the latent dimensions.

The structural modelling approach developed in this laboratory in other contexts has also been extended into another area of schizophrenia research - the analysis of PET data collected on a sample of normals and schizophrenics. This is a new approach to analysing these data that promises to be very powerful for describing the relationships among the functional areas of the brain. The unit of measure is the percentage of the glucose metabolism in a given area of the brain. An analysis of the normal subjects results in a precise description of the relationship of the areas in terms of coefficients stating the change in the glucose metabolism in a given area produced by change in each of the other areas. Because many coefficients will be zero it is possible to identify "pathways" through which stimulation of an area of the brain is propagated from area to area.

Next the structural analysis is applied to the schizophrenic subjects. The coefficients of the model applied to the schizophrenic subjects can then be compared to those of the normal subjects. It will be possible then to determine which "pathways" are shut down in schizophrenia, and which ones are activated that are not present in normals. This analysis can also be replicated with schizophrenic subjects under various therapies to determine the effects of these therapies with great precision.

SIGNIFICANCE OF THE RESEARCH:

The structural modelling approach, developed in this laboratory in previous research and extended to research in schizophrenia, promises to reveal patterns in both schizophrenia-like symptoms and the PET-scan measures of glucose metabolism in the brain that were not known before. And in either of these cases it is the precise quantification made possible by the method that generates the advantage.

PROPOSED COURSE OF FUTURE RESEARCH:

The latent table estimated in the latent class model of schizophrenia-like symptoms will be cross-classified against other variables available in the data set to see how the latent constructs vary by other properties and conditions of the subject population. The structural equation model of glucose metabolism in the brain as measured by the PET-scan will be applied to newly collected data that has higher resolution. This model will also be used to investigate the effects of different conditions on the glucose metabolism of the brain.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00680-06 LSES

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Work Experiences and the Deinstitutionalized Mentally Ill

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Elliot Liebow, Guest Researcher, LSES, NIMH

COOPERATING UNITS (if any)

None

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TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

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☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The objective of this exploratory, participant observation study is to examine the work experience of the deinstitutionalized mentally ill over time and to seek out ways in which job characteristics, symptoms, and social relationships interact with one another to effect the course of recovery from psychiatric disorder and reintegration into the community. Field work was carried out with residents of halfway houses, participants in community-based psychosocial and transitional work programs, and with "unattached" deinstitutionalized men and women.

Project Description:

Five years ago, Elliot Liebow, on detail to the Laboratory from the Extramural Program, began an exploratory, participant-observer study of the relationship between work experience and recovery from mental illness. The goal of this research was not to test hypotheses but rather to grasp the dynamics of the interaction between work experiences and recovery from mental illness.

In 1984, while still collecting data on deinstitutionalized persons in halfway houses and psychosocial programs in Montgomery County, Md., Liebow was stricken by two successive major illnesses. He retired on disability in September of that year but remained as a guest researcher in order to try to salvage some of the data he had already collected. These were somewhat too thin to serve their original purposes but were potentially useful nonetheless.

In November of 1984, Liebow began collecting data as a participant observer in a shelter for homeless women in Rockville. Many of the two dozen women who are "regulars" (in the sense that they stay at the shelter night after night, month after month) as well as the more casual users who come for a night or two, have a history of mental illness and/or institutionalization. Through these participant observation experiences, Liebow plans to contrast the dynamics and outcomes of two post-institutionalization life-styles: (a) the highly structured, tightly supervised group living of halfway houses and psycho-social day programs versus (b) the relatively unstructured, free floating life style of shelters and soup kitchens.

This research will focus primarily on the two dozen women who are "regulars" at the shelter. Liebow has now followed them intensely for more than 24 months. Data collection is completed, coding categories have been developed, and analysis and writing are underway.

Significance of the Research:

This project is directly pertinent to our understanding of rehabilitation of the deinstitutionalized mentally ill.

Proposed Course of Research:

Formal data collection has been completed. The investigator continues to work on the analysis and writing up of this data as a Guest Researcher at a moderate pace.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00681-02 LSES

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Reciprocal Effects of Self-Esteem and Depression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Rosenberg, Guest Researcher, Laboratory of Socio-environmental Studies, NIMH

OTHER: C. Schooler

Acting Chief

LSES

NIMH

C. Schoenbach

Social Science Analyst

LSES

NIMH

COOPERATING UNITS (if any)

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TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

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☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although low self-esteem has been shown to be associated with a number of non-psychotic disorders among children, adolescents, and adults, the causal connection among these variables has not been established. In order to do so, we plan to make use of a longitudinal data set. This is the Youth in Transition study, a five-wave national probability sample of adolescents. Through the use of instrumental variables and structural equation models, we will seek to specify the reciprocal effects of global self-esteem and a number of mental health and behavioral variables.

Project Description:

The aim of this project is to explore the reciprocal effects of global self-esteem and selected mental health and behavioral measures. Past research dealing with the relationship between self-esteem and other variables has been unable to answer the questions: Which is cause and which is effect (or, to be more exact, to what extent does each variable affect the other?).

Using a longitudinal data set consisting of a probability sample of 1886 male adolescents, we have explored the reciprocal effects of self-esteem and delinquency, school marks, and depression. To do this we have estimated full information linear structural equation models of these reciprocal effects. The results show that while low self-esteem fosters delinquency (-.19), delinquency reciprocally raises self-esteem (+.10). The relationship between self-esteem and school marks is not reciprocal. School marks do have a significant import on global self-esteem (+.14) but self-esteem does not have a significant effect on school marks (+.07). Finally, both self-esteem and depression have significant effects upon one another, but the effect of depression on self-esteem (-.25) is stronger than the effect of self-esteem on depression (-.16).

Significance of the Project:

This project has theoretical, methodological, policy, and therapeutic implications. The findings concerning the reciprocal effects of self-esteem and delinquency lend support to self-enhancement theory and frustration and aggression theory. The findings concerning self-esteem and school marks are consistent with self-attribution theory but not self-efficacy theory. These data are also of methodological interest because they show that the causal connections between the variables can be extremely complex. Whereas high self-esteem reduces delinquency, high delinquency enhances self-esteem. The two variables thus have countervailing effects. With respect to self-esteem and school marks, the effect is unidirectional; marks affect self-esteem but self-esteem does not affect marks. Finally, with respect to the relationships between self-esteem and depression, each variable significantly affects the other. All three reciprocal-effects analyses, then, reveal a different causal pattern. Third, these findings have policy relevance. Our data support policy recommendations attempting to forestall delinquency by enhancing self-esteem but do not support policies that attempt to improve school performance by raising self-esteem. Finally, the data suggest that one way to improve self-esteem is to reduce depression, but that one can also reduce depression by enhancing self-esteem.

Proposed Cause of Project:

Our initial success in solving these structural equation models encourages us to investigate other causal questions involving self-esteem. One of these deals with whether the parts are responsible for the whole or the whole is

responsible for the parts. For example, is the adolescent boy's academic self-concept responsible for his global self-esteem or vice versa. The causal status of other specific self-concept components will also be investigated. We also intend to explore the causal connection between self-esteem and parent-child relations. Research shows that adolescent self-esteem is powerfully associated with relationships with parent. Although the parent-child relationships are characteristically treated as the causal variable, it is possible that the opposite causal effect may also exist. Low self-esteem may be accompanied by certain personality dispositions (e.g., low self-disclosure, hostility) that have the effect of disturbing and alienating parents. These and other reciprocal effects analyses will be conducted in order to gain a better understanding of the causal dynamics associated with low self-esteem.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00682-02 LSES

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Environmental Determinants of Cognitive Functioning

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. Caplan, Staff Fellow

LSES

NIMH

OTHER: C. Schooler, Acting Chief

LSES

NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.00

PROFESSIONAL:

.75

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Results indicated that the effects of training organization were mediated by practice complexity: organization impaired performance after simple practice, and benefited performance after complex practice.

Project Description:

This research project investigates the ways in which environmental complexity affects cognitive functioning. Data collected by Melvin Kohn and Carmi Schooler over the last 20 years have demonstrated that individuals from complex environments demonstrate enhanced cognitive functioning, particularly in the area of intellectual flexibility. This series of studies is designed to shed light on the psychological mechanisms underlying these effects.

The first experiment in this project involved training people in the use of a microcomputer drawing program under different conditions of training organization and practice complexity. During training, instructions were presented in either a random or organized order, and either with or without an analogical model of the software package. Practice trials varied in both visual and logical complexity. Following training and practice, subjects' understanding of the package, as well as their ability to solve a drawing problem in several different ways (a measure of intellectual flexibility), were assessed. The results indicated that the effects of the model and of organization experienced during training depended on the complexity of the subsequent practice trials. In contrast with much of the cognitive psychology literature, which suggests that such manipulations are almost universally beneficial in learning, we found that the analogical model and organization help performance when an individual's experience in the domain has been complex, but can actually hurt performance when the experience has been simple. In addition, we found that individual differences in pre-existing skills or experiences can affect people's early learning of a new domain. However, as individuals gain experience in the new domain, differences in competence become less dependent on such background variables, and become more a function of the nature of the learning experience.

Data from the complete sample of 128 individuals have been analyzed, as have data from an additional 32 individuals who participated in a followup study conducted in the last year. The results of the major study have been written up and submitted for publication.

Significance of the Research:

The results of this project have implications both for Schooler's (1984) theory of the psychological effects of complexity, and for cognitive psychology in general. With respect to Schooler's theory, the results suggest that complexity does indeed affect the nature of people's cognitive functioning, and that it may in fact mediate the effects of the type of training methods commonly used as "teaching aids". Such aids may, in fact, only enhance learning under relatively simple conditions, and may actually impair learning in more complex learning situations. The project also addresses a number of important questions frequently ignored by mainstream

cognitive psychologists. Because they tend to study learning and problem-solving using tasks of low ecological validity, and relatively short training periods, they know little about learning and problem-solving in real-life domains. In addition, because most cognitive psychologists ignore the effects of individual differences on learning and problem-solving, we know relatively little about the effects of such variables. The results of this study will help to fill these gaps in our knowledge of learning and problem-solving.

Proposed Course of Research:

The results of this project to date pose an obvious question: how does stimulus organization impair learning and problem-solving under complex conditions, but enhance them under simple conditions? The next step in this research project involves designing and conducting studies to test hypotheses about the mechanism(s) which may underlie these findings.

Publications:

Schooler C. Social structural effects and experimental situations: mutual lessons of cognitive and social science. In: Schaie KW, Schooler C, eds. Social structure and aging. Hillsdale: Erlbaum, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00683-01 LSES

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Social and Cognitive Aspects of Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. Schooler, Acting Chief, Laboratory of Socio-environmental Studies, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.25

PROFESSIONAL:

.25

OTHER:

1.00

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The purpose of this project is to use the techniques of experimental psychology to investigate the nature of schizophrenics' social and cognitive functioning. One question being addressed is that of the effect of social situations on schizophrenics' performance. This is done in an experiment where schizophrenics perform a perceptual task in which a computer presents stimuli and evaluates response accuracy. Feedback is provided either by the experimenter or by the computer and subjects' responses to the two types of feedback are compared.

Plans are also underway to study the cognitive functioning of schizophrenics by examining their performance in experimental situations in which normally effective cognitive processes actually impair the performance levels of normal individuals. This will be done in order to see whether possible deficits in relevant aspects of schizophrenics' cognitive functioning might permit their performance level to surpass that of normals.

Project Description:

This new project of the LSES represents an attempt to use the techniques of experimental psychology to examine various aspects of the schizophrenic process. The examination of the first of these aspects--schizophrenic social dysfunction--is already underway. With the helpful collaboration of the NIMH Neuropsychiatric Research Hospital at St. Elizabeths, Schooler and Roberts have begun data collection in an experiment exploring the nature of the social dysfunction that seems to characterize schizophrenics. Earlier work in the Laboratory and elsewhere has consistently shown that not only are schizophrenics prone to avoid social interaction, but that they show a decrement in cognitive functioning as the intensity of social interaction increases. The reason for such social dysfunction remains unknown. It is plausible that the disruption that schizophrenics seem to experience in social situations may not result from the specifically human characteristics of the others. Instead, the generally complex nature of social situations may leave schizophrenics in a state of information overload. The study begun this year tests this possibility by examining schizophrenics' functioning in an experiment in which the social and non-social conditions are equated in their degree of cognitive complexity. This is done by using the computer's ability to both present stimuli and to evaluate response accuracy to compare schizophrenics' performance on a perceptual task when performance accuracy feedback is presented by a person or a computer.

Planning is currently underway for a second series of experiments with the dual purposes of investigating the cognitive effects of schizophrenia and the parameters of normal cognitive functioning. This approach combines two types of research: 1) studies in which the cognitive functioning of normals is adversely affected when certain forms of relevant information are called to their attention 2) research investigating how the cognitive functioning of schizophrenics is affected by their poor cognitive organization and how under some conditions this deficit may make their performance more efficient than that of normals. Juxtaposing these two types of studies on normal and schizophrenic samples should add substantially to our knowledge of both the schizophrenic disease process and normal cognitive functioning.

Significance of the Research:

There are both practical and theoretical reasons for attempting to gain an understanding of the nature of the social decrement in schizophrenia. From a practical point of view, knowing whether the decrement in cognitive processing that seems to characterize schizophrenics' functioning in social situations is at least partly due to the general level of complexity that typifies such situations or whether this cognitive disruption is a specific reaction to interacting with or even the mere presence of other people is an important consideration that must be taken account in deciding how to

try to improve schizophrenics' psychosocial adjustment. Such knowledge is also important in gaining an understanding of the disease process itself.

The experimental cognitive studies being considered are important for increasing our understanding of both normal and schizophrenic psychological functioning. Their approach is to examine whether schizophrenics' performance is similarly affected by those situations in which normals' performance is disrupted by the presentation of various types of structured information. If the schizophrenics' functioning is not so disrupted, it would suggest that they have some form of decrement in an area relevant to the processing of the potentially disruptive information. Following such a procedure has the advantage over most other studies of schizophrenic cognitive functioning in that the schizophrenics' possible decrement in functioning would become apparent through relatively better performance on the experimental task. Such a pattern of results would rule out the possibility that the findings are due to some type of generalized motivational or attentional dysfunction and at the same time point to the disruption of a relatively specific cognitive process. The very specificity of such disruption may well serve as an aid to the mapping of normal cognitive processes--one of the major tasks presently facing basic cognitive research. Thus, the proposed research has the potential of adding substantially to our understanding of the nature of both normal and psychopathological cognitive functioning.

Proposed Course of Further Research:

The recently originated investigation of the social aspects of schizophrenic psychopathology will be continued and the examination of schizophrenic cognitive processes presently being planned will be begun.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00684-01 LSES

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Representation of Semantic Categories

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. Caplan, Staff Fellow

LSES

NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

,50

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to investigate the nature of semantic category representation, and its effects on category-based reasoning. A series of experiments were conducted to test a model which makes a distinction between intrinsic and extrinsic features. The model successfully predicted performance in tasks assessing: 1) membership gradient, 2) class inclusion, and 3) linguistic contrast.

Significance of the Research

The model which is being developed and tested in this research project has so far been able to explain a number of empirical phenomena which no other single theory of categorization has been able to explain. In addition, it does so by positing a straightforward effect of a variable not previously considered in any detail by previous researchers - the nature of the features involved in category representation. It therefore represents a significant advance for theories of knowledge representation in general, and of natural language categories in particular.

Proposed Course of Research:

The paper which is currently in preparation will soon be completed and submitted for publication. In addition, future studies testing other aspects of the model (e.g., its implications for how people use categories in reasoning, and for the use of metaphor) are being planned.

Publications:

Caplan LJ, Barr RA. A comparison of context effects for typicality and category membership ratings. Proceedings of the tenth annual conference of the cognitive science society. Hillsdale: Erlbaum, in press.

Barr RA, Caplan, LJ. Category representations and their implications for category structure. Memory & Cognition 1987;15:397-418.

Project Description:

The study of the mental representations of semantic categories has long been a central area in cognitive psychology. Categories are devices by which knowledge of the past can be brought to bear upon the present, and are fundamental to an understanding of language, memory, problem-solving, and decision-making. According to the traditional view in philosophy and psychology (as well as that of most laypeople), natural language categories are represented by sets of features which are necessary and sufficient for category membership. In recent years, however, a number of findings have challenged this view: in particular, many researchers have demonstrated that the degree to which members are judged to be typical of categories varies from member to member, and may change across contexts even for a given member.

This project represents a continuation of an earlier line of research conducted with Robin Barr. It involves the development and testing of a model of category representation which maintains the essence of traditional theories, i.e., the proposal that category membership is determined by sets of necessary and sufficient features. However, it proposes that there are two kinds of features: intrinsic and extrinsic features. Intrinsic features are true of a category member in isolation (e.g., "has a tail" may be considered an intrinsic feature of a dog). Extrinsic features are relations between a category member and some other entity (e.g., "eats meat" may be considered an extrinsic feature of a dog). The model explains many of the findings in recent studies of categorization, and makes predictions about previously unstudied aspects of category-based reasoning.

The project has involved some re-analyses of the work of previous investigators, and a series of experiments testing the predictions of the model. In the last year, two studies were conducted to determine whether categories represented by intrinsic features differed from those represented by extrinsic features. The major study involved an investigation of the nature of context effects on judgments of category membership and category typicality as a function of the nature of category representation. In this experiment, subjects were asked to rate both category typicality and category membership of category members presented in a variety of contexts. The results indicated that context effects were more likely to be observed for typicality judgments than for membership judgments, and were more pronounced for extrinsically represented categories than for intrinsically represented categories. These results had been predicted by the model. This study has been accepted for publication (see below), and is being included in a larger, multi-experiment paper currently in preparation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00424-13 LCB
PERIOD COVERED October 1, 1987 through September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biologically Active Peptides in the Brain		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I.s Michael J. Brownstein, Chief, Laboratory of Cell Biology, NIMH T. Bonner, Special Expert, Laboratory of Cell Biology, NIMH C. Gerfen, Sr. Staff Fellow, Laboratory of Cell Biology, NIMH M. Palkovits, Visiting Scientist, Laboratory of Cell Biology, NIMH C. Weinberger, Sr. Staff Fellow, Laboratory of Cell Biology, NIMH W.S. Young, Sr. Staff Fellow, Laboratory of Cell Biology, NIMH (see attached)		
COOPERATING UNITS (if any) Good Samaritan Hosp., Portland, Oregon; Duke U. Med. School; MD, NIDDKD, Purdue Univ.; JHU; LNC, NINCDS; Westminster Hosp., London		
LAB/BRANCH Laboratory of Cell Biology		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 10	PROFESSIONAL: 0.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p> The structures, distributions, and functions of molecules of importance in the nervous system are being studied. Several cDNAs that encode neurotransmitter receptors have been isolated. The distributions of mRNAs to which these DNAs are complementary have been determined by means of in situ hybridization histochemistry (ISHH) as have the distributions of other mRNAs (e.g., thyroid hormone receptor, G-proteins, phospholipases). ISHH has also been used to examine the effects of physiological and pharmacological manipulations on specific mRNA levels in the CNS. Expression of receptor cDNAs has allowed functional studies to be carried out--especially studies of receptor coupling to second messenger systems. </p>		

Other Professional Personnel Engaged on Project

J. Axelrod	Guest Researcher	LCB, NIMH
N. Barmack	Sr. Scientist	Good Samaritan Hospital
P. Blackshear	Assoc. Professor	Duke Univ. Med. School
D. Bradley	Guest Researcher	LCB, NIMH
M. Brann	Staff Fellow	MD, NIDDKD
N. Buckley	Visiting Fellow	LCB, NIMH
J. Dixon	Professor	Purdue Univ.
C. Felder	Staff Fellow	LCB, NIMH
J. Hadreen	Assoc. Professor	JHU
B. Hoffman	Guest Researcher	LCB, NIMH
S. Horváth	Visiting Fellow	LCB, NIMH
K. Koller	Staff Fellow	LCB, NIMH
K. Kusano	Visiting Associate	LNC, NINCDS
S. Lightman	Reader in Medicine	Westminster Hosp., London
S. Lolait	Guest Researcher	LCB, NIMH
L. Mahan	Staff Fellow	LCB, NIMH
L. Matsuda	Guest Researcher	LCB, NIMH
E. Mezey	Visiting Scientist	LCB, NIMH
A.-M. O'Carroll	Visiting Fellow	LCB, NIMH
N. Rance	Postdoctoral Fellow	JHU
A. Young	Chemist	LCB, NIMH

Project Description:Cloning and sequencing of cDNAs and genomic DNAs

T. Bonner, A. Young, N. Buckley, and M. Brann have added a fifth subtype to their list of muscarinic receptors.

L. Matsuda and T. Bonner have isolated two cDNA clones that encode proteins structurally very similar to the known G-protein binding receptors. They are presently attempting to find the ligands that bind to these putative receptors.

B. Hoffman, having cloned a partial cDNA for the serotonin 5-HT_{1c} receptor, has now isolated full-length clones encoding the 5-HT_{1c} and 1a receptors. She hopes to isolate other 5-HT receptor cDNAs and to use these to study 5-HT pharmacology.

K. Koller, K. Kusano, and M. Brownstein have shown that mRNA extracted from AR42J cells encodes a cholecystokinin receptor. The receptor mRNA is about 3Kb in size. A cDNA library prepared from the positive mRNA fraction is being used in an attempt to clone the receptor.

S. Lolait, A.-M. O'Carroll, and L. Mahan have isolated cDNAs encoding several α and β subunits of the GABA-A receptor.

C. Weinberger has shown that lambda expression libraries can be screened for the presence of cDNAs that encode molecules such as the thyroid hormone receptor with radiolabeled ligands. He hopes to use this method to identify the dioxin receptor gene.

M. Brownstein and J. Dixon have isolated a full-length cDNA that encodes the rat carboxypeptidase H processing enzyme.

Dr. Brownstein has constructed a novel plasmid vector based on the original Okayama-Berg vector that should be useful for expression cloning in yeast, mammalian cells, and *Xenopus* oocytes.

Studies of receptors and second messengers

Drs. Bonner, Buckley, Brann, Felder, and Axelrod have shown that cells transfected with cDNAs encoding muscarinic receptors generate arachidonic acid when they are stimulated with acetylcholine. The various muscarinic receptors were found to differ in their ability to couple to G-proteins and second messenger systems (i.e., systems that increase arachidonic acid, cAMP, and phosphatidylinositol turnover). Arachidonic acid release by m1, m3, and m5 receptor stimulation seems to result from activation of phospholipase A₂.

Dr. Bonner and his colleagues are examining genetically altered muscarinic receptors in order to determine the structural basis of their interactions with second messenger systems.

Anatomical studies

Dr. Mezey has used immunocytochemistry and *in situ* hybridization histochemistry (ISHH) to demonstrate phenylethanolamine-N-methyl transferase producing cells in the amygdala.

Drs. Horváth and Palkovits have shown by means of immunoelectron microscopy that somatostatin- and growth hormone releasing hormone-positive cells interact with one another. They have also begun to characterize the CNS lesion produced by N-methylamino-L-alanine in rodents. This neurotoxin is thought to be responsible for the Guamanian ALS-Parkinson's Disease-dementia complex.

Drs. Young and Lightman have continued to study the response of rat hypothalamic mRNAs to stress, opiate withdrawal, lactation, and hyperosmolality by means of ISHH. Drs. Young and Rance are using the latter technique to examine the normal anatomy of the human hypothalamus, and Dr. Young is studying the role of CRF in the inferior olivary nucleus in regulating eye movements in collaboration with Dr. Neal Barmack. In addition, he is working with Drs. Hadreen and Blackshear in studies of Huntington's Disease and regulation of ornithine decarboxylase, respectively.

Dr. Gerfen has demonstrated that the compartmental organization of the corticostriatal system is dependent on the laminar organization of the cerebral cortex. This suggests that a major function of the patch and matrix system is related to the manner in which inputs from the allo- and isocortical areas are integrated by the basal ganglia.

Dr. Gerfen has also examined the effects of nigrostriatal lesions and dopamine agonists or antagonists on second messenger systems in the striatum by means of ISHH. There were specific changes in mRNAs encoding G_s (but not G_i or G_o), protein kinase C (PKC1, 2, and 3) and phospholipase C (PLC I, II, and III). There were changes in dynorphin, enkephalin, and substance P mRNAs as well.

C. Weinberger and D. Bradley have used in situ hybridization histochemistry to map the distribution of thyroid hormone receptors in the CNS.

Significance to Biomedical Research

Nerve cells use chemical "transmitters" to communicate with one another and with other target cells. Changes in transmitter biosynthesis, release, and/or metabolism have been suggested to result in nervous and mental disorders. Death of dopaminergic neurons in the substantia nigra, for example, is associated with the symptoms of Parkinson's disease. In the last ten years the number of putative neurotransmitters has increased by a factor of four or five. Most of the newly detected chemical messengers are peptides. Our knowledge of the anatomy, physiology and pharmacology of peptidergic neurons is comparatively incomplete at present; indeed, it is clear that many biologically active peptides remain to be isolated and characterized.

The work outlined above is principally devoted to improving our understanding of cells. To the extent that we understand these cells, we can formulate better hypotheses about their role in causing disease.

Proposed Course

The work outlined above is still in progress and will be continued.

Publications

Koller KJ, Wolff RS, Warden MK, Zoeller RT. Thyroid hormones regulate levels of thyrotropin-releasing hormone mRNA in the paraventricular nucleus. *Proc Natl Acad Sci USA* 1987;84:7329-33.

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hypertensive rats. In: Brenner BM, Laragh JH, eds. *Advances in atrial peptide research*; vol 2. New York: Raven Press, 1988;534-8.

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Gerfen CR, Baimbridge KG, Thibault J. The neostriatal mosaic: III. Biochemical and developmental dissociation of dual nigrostriatal dopaminergic systems. *J Neurosci* 1987;7:3935-44.

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Hokfelt T, Foster GA, Johansson O, Schultzberg M, Holets V, Ju G, Skagerberg G, Palkovits M, Skirboll L, Stolk JM, U'Prichard DC, Goldstein M. Central phenylethanolamine-N-methyltransferase-immunoreactive neurons: distribution, projections, fine structure, ontogeny, and coexisting papers. In: Stolk JM, U'Prichard DC, Fuxe K, eds. *Epinephrine in the nervous system, Part II*. New York: Oxford University Press, 1988;10-32.

Kerdellhue B, Parnet P, Lenoir V, Schirar A, Gaudoux F, Levasseur MC, Palkovits M, Blacker C, Scholler R. Interactions between 17 beta-estradiol and the hypothalamo-pituitary beta-endorphin system in the regulation of the cyclic LH secretion. *J Steroid Biochem* 1988;30:161-8.

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PM, Hsia YE, Gusella JF. Von Hippel-Lindau disease maps to the region of chromosome 3 associated with the renal cell carcinoma. *Nature* 1988;332:268-9.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00422-17 LCB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropharmacology of Circadian Rhythms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Zatz Section Chief SBP, LCB, NIMH

Others: N. Harrison Visiting Fellow LNP, NINCDS

COOPERATING UNITS (if any)

LNP, NINCDS

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Section on Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.1

PROFESSIONAL:

1.1

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Circadian rhythms and environmental lighting regulate a number of endocrine and behavioral functions. Dispersed chick pineal cells remain rhythmic and responsive to light in culture. Light has two apparent effects on the melatonin rhythm displayed by these cells: the first is an acute inhibition of melatonin output, the second is entrainment of the underlying pacemaker. Pertussis toxin, which acts to block the function of transducin and G_i , blocked the acute effects of light but not its entraining effects. There must, therefore, be at least two mechanistic pathways for photoendocrine transduction in the chick pineal. The mechanisms of phototransduction appear to differ from those in retinal rod cells. Light and calcium influx appear to regulate melatonin production (acutely) via cyclic AMP. The mechanisms of photoentrainment remain unknown.

Project Description

Objectives: To elucidate the biochemical mechanisms and neuropharmacology of circadian rhythms; to elucidate the mechanisms by which light suppresses and entrains melatonin rhythms.

Methods: Biochemical, pharmacologic, electrophysiologic, cell culture, and radioactive trace techniques.

Major Findings: Several laboratories have demonstrated the persistence of photosensitive rhythms related to melatonin secretion in cultured chick pineals. We have developed a system using dispersed chick pineal cells in static culture, which displays a rhythm of melatonin release for at least two weeks under cyclic lighting conditions, and for at least 4 cycles under constant red light. Using a rapid and specific extraction assay for the ^{14}C -melatonin formed (from ^{14}C -tryptophan) and secreted by these cells, we have examined the effects of perturbations on the amplitude, period, and phase of the melatonin rhythm. With this approach, simultaneous comparisons of the effects of multiple, independent perturbations on virtually identical, cycling, photosensitive cells can be made.

Light has two apparent effects on this melatonin rhythm; the first is an acute inhibition of melatonin output, the second is entrainment of the underlying pacemaker. These two effects could be mediated by the same or different mechanisms. Pertussis toxin, which acts to block the function of transducin and certain other G-proteins, blocked the acute effects of light on chick pineal cells, but not the ability of light pulses to induce phase-dependent phase shifts of the rhythm. There must, therefore, be at least two mechanistic pathways by which light affects chick pineal melatonin production. Transducin or other pertussis toxin-sensitive G-proteins would appear to be involved in the acute effects of light on the melatonin-synthesizing apparatus, but not in the effects of light on the circadian pacemaker which generates the melatonin rhythm. We showed previously that norepinephrine, acting through an α_2 -adrenergic receptor, mimics the acute suppressive effect of light, but does not cause phase shifts in the rhythm of melatonin release. Cyclic AMP analogs and stimulants increased melatonin output without affecting the underlying pacemaker. Taken together, these results support the inference that reduction in cyclic AMP levels, presumably mediated by the pertussis-toxin sensitive GTP-binding protein G_i mediates the acute suppression of melatonin production by light or norepinephrine. This mechanism differs from the mechanism of phototransduction in retinal rod cells.

The nocturnal increase in melatonin output requires extracellular calcium and can be suppressed both by inorganic calcium channel blockers (Co^{++} or Mn^{++}) and by dihydropyridine antagonists; conversely, melatonin output was enhanced by the calcium channel "agonist" Bay K 8644. These data suggest that calcium influx is important in the regulation of melatonin

production. We showed previously, by electrophysiologic techniques, the presence of "L-type" voltage sensitive calcium channels in chick pineal cells. Evidence for the presence of a cation channel that is not voltage sensitive and carries inward current, which is reminiscent of the photosensitive cation channel in retinal cells, has been obtained. In order to determine whether calcium influx regulates the circadian pacemaker in chick pineal cells, we tested the ability of agents to phase shift the melatonin cycle in constant red light. Four or eight hour pulses of NTR, BK, Co^{++} , or low Ca^{++} did not appreciably alter the phase of subsequent melatonin cycles. Neither did BK interfere with phase shifts induced by light pulses. Mn^{++} pulses did induce phase-dependent phase shifts, but, unlike those evoked by light or dark pulses, these were all delays. Such effects of Mn^{++} in other systems have been attributed to, and are characteristic of, "metabolic inhibitors." On balance, the results fail to support a prominent role for calcium influx in regulating the pacemaker underlying the circadian rhythm in chick pineal cells. We then asked about the relationship between calcium influx, cAMP, and light, in the acute regulation of melatonin production. Neither nitrendipine nor low external calcium reduced melatonin output in the presence of 8BrcAMP. Norepinephrine, however, did lower melatonin release in the presence of Bay K 8644. These results suggest that calcium influx regulates melatonin synthesis via cAMP. Addition of 8BrcAMP blocked the acute inhibitory effect of light, but Bay K 8644 did not. These results suggest that light need not act via calcium influx to regulate cAMP; rather both light and calcium influx act on cAMP to regulate melatonin output.

Significance to Biomedical Research: Circadian rhythms occur in hormone levels, activity, mood, etc. and are primarily regulated by light-dark cycles. The mechanisms generating and regulating circadian rhythms are of broad clinical and biologic interest. This photosensitive cultured cell system, with its biochemically measurable output, has unique advantages for the investigation of biochemical mechanisms regulating phototransduction and circadian rhythmicity.

Proposed Course of Project: Pharmacologic agents acting on the pacemaker will be sought; agents acting on sodium channels and exchange mechanisms, protein synthesis, and retinoids will be tested for direct effects on the pacemaker and for interactions with the effects of light. If feasible, dynamic regulation of the photopigment, transducin, and G_i , will be sought at the protein and mRNA levels. The photopigments regulating melatonin and the pacemaker will be sought through their chromophores. Mechanisms involved in regulation of adenylate cyclase by light and calcium influx will be explored.

Publications:

Zatz M, Mullen DA, Moskal JR. Photoendocrine transduction in cultured chick pineal cells: Effects of light, dark, and potassium

on the melatonin rhythm, Brain Res 1988;438:199-215.

Zatz M. Pondering the pineal in chick vs. rat. In: Sandler M, ed. Proceedings of the 6th International Catecholamine Symposium. New York: A R Liss (in press).

Zatz M, Mullen DA. Norepinephrine, acting via adenylate cyclase, inhibits melatonin output but does not phase-shift the pacemaker in cultured chick pineal cells, Brain Res 1988;450:137-43.

Zatz M, Mullen DA. Photoendocrine transduction in cultured chick pineal cells. II. Effects of forskolin, 8-bromocyclic AMP, and 8-bromocyclic GMP on the melatonin rhythm, Brain Res (in press).

Wallingford JC, Zatz M. A novel photopigment candidate in membranes of cultured chick pineal cells, Exp Eye Res (in press).

Zatz M, Mullen DA. Two mechanisms of photoendocrine transduction in cultured chick pineal cells: pertussis toxin blocks the acute but not the phase-shifting effects of light on the melatonin rhythm, Brain Res (in press).

Zatz M, Mullen DA. Does calcium influx regulate melatonin production through the circadian pacemaker in chick pineal cells? Effects of nitrendipine, Bay K 8644, Co^{++} , Mn^{++} , and low external Ca^{++} , Brain Res (in press).

Zatz M. Relationship between light, calcium influx, and cAMP in the acute regulation of melatonin production by cultured chick pineal cells, Brain Res (in press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00429-09 LCB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemistry of Membranes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Zatz Section Chief SBP, LCB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Section on Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

1) Previous work explored the mechanisms by which lithium causes ACTH secretion from anterior pituitary cells. It is hypothesized that this effect is mediated by lithium's action on $\text{Na}^+/\text{Ca}^{++}$ exchange mechanisms. Further work on this problem was postponed.

2) Previous work demonstrated the acylation of rhodopsin by long chain fatty acids in vivo and in vitro. Further work on the role of this new class of post-translational modification in receptor function was postponed.

Project Description:

Objectives: 1) To determine the role of membrane ion exchange mechanisms in the effects of lithium. 2) To elucidate the nature and function of protein acylation.

Methods: Biochemical, pharmacologic, enzymatic, cell culture, and radioactive trace techniques.

Major Findings: None this year.

Significance to Biomedical Research: Elucidation of an action of lithium on cell function and membrane processes could shed light on the therapeutic actions of lithium. Acylation of membrane proteins provides a mechanism for posttranslational modification of the lipophilicity of receptors, ion channels, etc., which could alter their functional interactions with cell membranes, other proteins, or signal molecules.

Proposed Course of Project:

1) Lithium's interactions with cellular ion exchange mechanisms will be explored. 2) The role of acylation in regulation of membrane receptor function will be explored.

Publications:

Zatz M, Reisine T. Corticotropin (ACTH) secretion. In: Johnson FN, ed. Lithium therapy monographs, vol II, Lithium and the endocrine system. New York: Karger Medical Publishers, 1988;147-58.

Reisine T, Zatz M. Interactions between lithium, calcium, diacylglycerides and phorbol esters in the regulation of ACTH release from AtT-20 cells, J Neurochem 1987;49:884-9.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00434-07 LCB
PERIOD COVERED October 1, 1987 through September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Molecular Mechanisms of Receptor-Mediated Signal Transduction</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Julius Axelrod Guest Researcher LCB, NIMH Others: R. Burch Guest Researcher LCB, NIMH B. Conklin Guest Researcher LCB, NIMH C. Felder Staff Fellow LCB, NIMH C. L. Jelsema Guest Researcher LCB, NIMH A. L. Ma Biologist LCB, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Cell Biology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 5.0	PROFESSIONAL: 5.0	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Interleukin-1 (IL-2)</u> stimulated the release of <u>PGE₂</u> in <u>Swiss 3T3 cells</u>. Pretreatment of the cells with IL-2 enhanced the bradykinin stimulated release of <u>PGE₂</u> 10 fold. Similar enhancement was seen for bombesin and thrombin IL-2 induced <u>phospholipase A₂ cyclooxygenase</u>, and <u>GTPase</u> but did not alter receptor number or affinity. <u>Diacylglycerol</u> also stimulated phospholipase A₂ activity in the Swiss 3T3 cells. Unique <u>bradykinin receptors</u> unlike the previously described B₁ and B₂ classification were pharmacologically identified using several bradykinin analogs. These receptors were further characterized according to their differential coupling to phospholipase-C and phospholipase A₂ signal transduction systems. <u>Muscarinic receptors</u> m₁, m₃ and m₅ transfected into fibroblasts stimulated arachidonic acid release, inositolphospholipid turnover and cAMP accumulation. Muscarinic receptors m₂ and m₄ caused a decrease in cAMP accumulation. The receptor mediated release of arachidonic acid was distinct from <u>inositolphospholipid turnover</u>. PMA and carbachol caused a synergistic stimulation of arachidonic acid release, but PMA inhibited inositolphospholipid turnover. Dopamine-1 receptors stimulated inositolphospholipid turnover in <u>renal cortical plasma membranes</u>. This stimulation was independent of adenylate cyclase activity and appear to be linked to phospholipase-C via a cholera and pertussin toxin insensitive guanine nucleotide binding protein. A specific GTP-binding protein, transducin, has been demonstrated to function in the receptor-mediated activation of phospholipase A₂ and C in bovine retina. Both phospholipases appear to be under inhibitory control in this tissue. Kinases appear to play a role in the signal transduction of phospholipase A₂ and phospholipase C by modification of the G proteins that couple receptor activation to changes in effector systems within the cell. </p>		

Project Description:Interleukin-1 Amplified Receptor-Mediated Activation of Phospholipase A₂:

Julius Axelrod in collaboration with Ronald Burch (Nova Pharmaceutical) has shown that human recombinant interleukin-1-alpha and interleukin-1-beta stimulated prostaglandin E₂ (PGE₂) synthesis in 3T3 fibroblasts in a time and dose-dependent manner. Interleukin-1 was potent with a EC₅₀ of 0.5 pM using either the alpha or beta form. In cells that were pretreated with interleukin-1 for 24 hrs., PGE₂ synthesis in response to bradykinin, thrombin, and bombesin, was amplified many fold. Interleukin-1 treatment induced phospholipase A₂ and cyclooxygenase and GTPase but not phospholipase-C or prostaglandin E isomerase.

Pharmacological Identification of Two Bradykinin Receptors Linked Differentially to Phospholipase A and C Stimulation:

In collaboration with Ronald Burch and Julius Axelrod, Bruce Conklin completed his study of bradykinin receptors in fibroblasts and endothelial cells. This study showed that two bradykinin receptors could be distinguished through the use of several bradykinin analogues which do not fit the previously described B₁, B₂ bradykinin receptor classification. Furthermore, these two bradykinin receptors have been distinguished by differential coupling to phospholipase A₂ and C.

Coupling of Transfected Muscarinic Receptors to Phospholipase A and C:

In collaboration with Mark Brann, Noel Buckley, Tom Bonner, Christian Felder, Alice Ma and Julius Axelrod, Bruce Conklin has demonstrated that the stimulation of transfected muscarinic receptors can generate arachidonic acid as a second messenger. Muscarinic receptors m1-m4 were found to differ in their ability to couple G-proteins and second messenger systems, such as the release of archidonic acid, cAMP accumulation, and phosphatidylinositol (PI) turnover. Arachidonic acid release by the m1 and m3 receptors was shown to be regulated independently of PI turnover and cAMP accumulation. Differential effects of the phorbol ester, PMA and the phospholipase A₂ inhibitor, mepacrine on arachidonic acid release and PI turnover indicated that the main source of AA release mediated by m1, m3 muscarinic receptors was the activation of phospholipase A₂.

Christian Felder in collaboration with Bruce Conklin, Mark Brann, and Julius Axelrod, has shown that the novel muscarinic-5 receptor, which has been expressed in CHO cells, appears to be linked to phospholipase A₂ activation and arachidonic acid release.

Dopamine-1 Stimulated Phospholipase C:

Christian Felder in collaboration with Julius Axelrod and Pedro Jose (Georgetown University) has demonstrated that the stimulation of phospholipase C by DA-1 agonists is distinct from alpha-1 receptor stimulated phospholipase C in renal cortical plasma membranes. It was also shown that DA-1 agonists stimulated adenylate cyclase and phospholipase C independently, thus demonstrating a new transduction pathway for DA-1 receptors.

Significance to Biochemical Research:

Many drugs relieve depression and schizophrenia by interacting with neurotransmitters and their receptors. To fully understand the interaction of neurotransmitter and hormone receptors on cell processes requires a detailed knowledge of post receptor signal transduction systems. As more is learned of each second messenger system, it becomes clear that each pathway is a complex arrangement of multiple functional units allowing for greater specificity and regulation. In addition, there appears to be cross-talk between each individual transduction system providing for a higher level of regulation. The cloning and transfection of receptors into cells that do not normally express the receptors provides an ideal system to study signal transduction in more detail. There is increasing evidence that the immune system interacts with the nervous system. Our work on the effect of lymphokines on peptide receptors provides a biochemical link between these two systems.

Proposed Course of Project:

Future studies on the muscarinic receptors will focus on the cross talk between the receptor mediated stimulation of phospholipase A₂, phospholipase C, and adenylate cyclase. The m₁, m₃, and m₅ receptors transfected into chinese hamster ovary cells and A9 fibroblasts provide the ideal model system for studying these complex interactions since they stimulate all

three signal transduction systems. Julius Axelrod, Chris Felder, and Alice Ma will be working on this project in collaboration with Mark Brann.

Ron Burch's and Julius Axelrod's observation that IL-1 can augment the receptor mediated release of PGE₂ by bradykinin in Swiss 3T3 cells suggests that IL-1 may play a role in the regulation of signal transduction processes. Several Neural cell lines to be provided by Phil Nelson and Douglas Brenneman will be screened for IL-1 response. Preliminary results suggest that IL-1 enhances the carbachol stimulated release of arachidonic acid from chinese hamster ovary cells transfected with the m₅ receptor. It is not known if IL-1 enhances the arachidonic acid release for the m₁ and m₃ receptor. These studies will be expanded to determine the mechanism of the IL-1 response and determine if any IL-1 mediated responses occur in the brain. This project will be the collaborative effort of Julius Axelrod, Ron Burch, Phil Nelson, Douglas Brenneman and Chris Felder.

Role of G proteins in the receptor-mediated activation of phospholipase A₂ and phospholipase C:

Dr. Jelsema has previously demonstrated that in the rod outer segments of bovine retina, both phospholipase A₂ (PLA₂) and phospholipase C (PLase C) are under dual regulation by GTP-binding proteins. The retinal G protein, transducin, functions as the stimulatory G protein for both phospholipases while a pertussis toxin-sensitive G protein serves as the inhibitory G protein for PLase C and a cholera toxin-sensitive inhibitory G protein serves as the PLA₂-inhibitory G protein. The identity of these inhibitory G proteins is currently under investigation while the subunit specificity for the transducin-mediated effects on PLA₂ and PLase C has been demonstrated. In the activation of PLA₂, the $\beta\gamma$ subunits of transducin stimulate PLA₂ while the α subunit inhibits this effect, presumably by facilitating subunit reassociation. The mechanism for phospholipase A₂ stimulation by the $\beta\gamma$ subunits appears to involve the inactivation of a PLA₂ inhibitor rather than direct activation of the enzyme. In the modulation of PLase C, the transducin α subunit is stimulatory while the $\beta\gamma$ subunits are inhibitory in the absence of light whether or not the α subunit is present but have a stimulatory effect in the presence of light that is additive with the stimulatory effects of the α subunit. The mechanism for the α and $\beta\gamma$ -induced effects on PLase C are currently under investigation. Since the $\beta\gamma$

subunits are common to all G proteins, the modulation of phospholipase A₂ and C by these subunits has implications for all signal-transducing systems that employ G proteins.

Dr. Jelsema, in collaboration with S.-G. Rhee, has demonstrated the existence of three distinct PLase C isozymes in rod outer segments of bovine retina, each of which appears to be regulated either directly or indirectly by different G proteins as evidenced by the different effects of light, GTP γ S and pertussis and cholera toxin on the hydrolysis of phosphatidylinositol, phosphatidylinositol-4-phosphate and phosphatidylinositol-4,5-bisphosphate. A portion of this study is focused on the apparent translocation of specific PLase C isozymes in response to light. These studies, while revealing unexpected complexities, are important to understanding the regulation of this second messenger system.

Dr. Jelsema, in an initial collaboration with A.D. Ma (Univ. Michigan), examined the role of G protein subunits other than transducin in the modulation of phospholipase A₂ activity using a reconstituted system that consists of isolated, purified G protein subunits, porcine pancreas phospholipase A₂ and phosphatidylcholine vesicles. Modulation of enzyme activity by G proteins in this reconstituted system only occurred in the presence of a PLA₂ inhibitor. Studies in collaboration with Dr. C. Felder designed to isolate the membrane-bound phospholipase A₂ of the rod outer segments for reconstitution with the G protein subunits revealed that the enzyme, once partially purified, was fully active and not responsive to G proteins in the absence of the PLA₂ inhibitor calpactin.

Dr. Jelsema, in collaboration with Dr. D. Rausch, have discovered that nerve growth factor (NGF) inhibits PLA₂ while stimulating PLase C activity in PC12 pheochromocytoma cells. They are currently examining the effect that infection with ras or src has on these two phospholipases since these infections, similar to NGF treatment, lead to neurite extension. Since both ras expression as well as src-induced kinase activity have been implicated in the regulation of these two phospholipases, these studies should provide clues not only to the role of phospholipases in the mode of action of these oncogenes but also the role of phospholipases in neuronal development and in the action of NGF. The possibility that one of the isozymes of PLase C is responsive to activation by tyrosine kinases while other isozymes respond to G protein manipulation may explain how diverse

agents exert similar effects.

Dr. Jelsema, in collaboration with Dr. M. Jett (Walter Reed) has discovered that Staphylococcus toxin stimulates both PLA₂ and PLase C. Furthermore, the action may be similar to pertussis and cholera toxins since there does appear to be ADP-ribosylation with the toxin. The possibility that G proteins may serve as toxin substrates is currently being assessed. The possibility that another toxin may modulate phospholipase activities by ADP-ribosylation would greatly facilitate the identification of the G proteins involved in regulation of the phospholipases and is worthy of investigation.

Studies of protein kinase C modulation of GTP-binding proteins:

Dr. Jelsema, in collaboration with Drs. R. Kahn (NCI), S. Jakens (Alton Jones Cell Science Center, Lake Placid, New York) and A.D. Ma have previously analyzed the in vitro phosphorylation of the GTP-binding proteins G_s, G_i, G_o and transducin using 3 distinct isozymes of protein kinase C and cAMP-dependent protein kinase. Only the G protein α subunits were found to be substrates for the kinases, with the kinases exhibiting marked differences in substrate specificity. The functional effect of the G protein phosphorylation was examined in terms of the capacity of the G proteins to modulate phospholipase A₂ activity in a reconstituted system. In collaboration with Dr. Waldbillig (NEI) this study has recently been expanded to include G protein phosphorylation by insulin-like growth factor tyrosine kinase. These studies illustrate the possibility that desensitization of a receptor-mediated signal may also occur at the level of the G protein.

Publications

Axelrod J, Burch RM, Jelsema CL. Receptor-mediated activation of phospholipase A₂. Arachidonic acids and its metabolites as second messages in receptor-receptor interactions. In: Fuxe K, Agnati LF, eds. Wenner Green Instructional Series 48. London: Macmillan, 1987;298-397.

Jelsema CL, Axelrod J. Light activation of phospholipases in rod outer segments. In: Hudspeth JA, Macleish P, Margolis R, Wiesel T, eds. Sensory transduction, discussions in neuroscience; vol 4. Geneva: FESN, 1987;79-84.

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- Axelrod J, Burch RM, Jelsema CL. Receptor-mediated activation of phospholipase A2 via GTP-binding proteins: arachidonic acid and its metabolites as second messengers. *Trends Neurosci* 1988;11:117-23.
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- Conklin BR, Burch RM, Sterenka LK, Axelrod J. Distinct receptors mediate stimulation of prostaglandin synthesis by endothelial cells and fibroblasts. *J Pharmacol Exp Ther* 1988;244:646-9.
- Jelsema CL. Modulation of the phospholipase A2 activity of dark-adapted, transducin-poor rod outer segments of bovine retina by G-protein subunits, guanine nucleotides and protein kinases. In: FRedburn D, ed. Alan R Liss Inc. *Extracellular and Intracellular Messengers in the Vertebrate Retina* 1988;in press.
- Axelrod J. Following the trail of epinephrine from the periphery to the brain. In: Oxford Press Stolk JM, Prichard DC, Fuxe K. *The Central Nervous System*. 1988;in press.
- Conklin BR, Brann MR, Buckley NJ, Ma AL, Bonner, TI, Axelrod J. Stimulation of arachidonic release and inhibition of mitogenesis by cloned muscarinic receptor subtypes stably expressed in A9L cells. In: *Proc Natl Acad Sci* 1988;in press.
- Felder RA, Felder CC, Eisner BM, José PA. Renal dopamine receptors. In: McGrath B, Bell C, eds. *Macmillan*, London. 1988;in press.
- Felder CC, José PA, Axelrod J. The dopamine-1 agonist, SKF 82526, stimulates phospholipase C activity independent of adenylate cyclase. *J Pharmacol Exp Ther*; in press.

Burch RM, Axelrod J. Disassociation of bradykinin-induced prostaglandin formation from phosphatidylinositol turnover in Swiss 3T3 fibroblasts: Evidence for G protein regulation of phospholipase A2. In: Proc Natl Acad Sci USA; 1988;6374-8.

Karsenty G, Alquier, Jelsema CL, Weintraub BD. Thyrotropin induces growth and iodothyronine production in a human thyroid cell line without affecting cyclic AMP production. J Physiol in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02385-02 LCB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Control of Cell Differentiation, Growth and Transformation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H. Okayama	Visiting Scientist	LCB, NIMH
Others:	M. Eiden	Guest Researcher	LCB, NIMH
	A. Masuda	Visiting Fellow	LCB, NIMH
	K. Okazaki	Visiting Fellow	LCB, NIMH
	N. Okazaki	Guest Researcher	LCB, NIMH
	R. Toyama	Visiting Fellow	LCB, NIMH
	T. Yamashita	Visiting Associate	LCB, NIMH
	C. Chen	Biologist	LCB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cell Biology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.4

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mutant NPK cells that are no longer sensitive to TGF- β and EGF have been generated and isolated. These will be used to study the role of TGF- β in malignant transformation.

A cDNA has been isolated that causes chemically transformed Baby Hamster Kidney (BHK) cells to revert to normal phenotype. The mode of action of this recessive oncogene will now be investigated.

A highly efficient technique for transfecting the yeast, schizosaccharomyces pombe has been developed. The method will be used to clone cDNAs that complement mutant yeast that have defective cell cycles, DNA repair mechanisms, etc.

Recombinant viruses have been generated capable of infecting cells but not of replicating within them. These can safely and conveniently be used to study the interaction between viruses and their receptors. In addition, the recombinant viruses may be an efficient means of introducing cloned genes into infectable cells.

Project Description:

Objectives: To elucidate the molecular mechanism of cell differentiation, growth control and malignant transformation: cloning of cellular oncogenes and genetic elements involved in transformation, and genes for growth and differentiation factor signal transduction pathways.

Methods employed: Recombinant DNA, molecular cloning, cell culture, and gene analysis techniques.

Major findings:

Studies of cellular growth, development, and transformation

Drs. Masuda and Okayama have generated a number of mutant NRK cells that are no longer sensitive to TGF- β or EGF. They hope to use these mutants to characterize the components of the TGF- β transduction system and to understand the role of TGF- β in malignant transformation.

Expression of the α subunit of human chorionic gonadotropin in the HeLa cells correlates well with tumorigenicity of these cells. The former is suppressed when transformed HeLa cells are fused with normal cells. Drs. Toyama and Okayama are constructing plasmids containing the HCG promoter upstream of a selectable marker. They plan to introduce these plasmids into transformed HeLa cells and then introduce a cDNA library from normal cells. Then by selecting against cells in which the HCG promoter is still active they hope to isolate novel recessive oncogenes.

Drs. Yamashita and Okayama have transfected v-Ki-ras-transformed NIH 3T3 cells with a human cDNA library. They are attempting to isolate colonies of cells that have reverted to normal (non-transformed) phenotype. They hope in this way to find human cDNAs encoding proteins with ras-suppressing activities.

Drs. Armbruster and Bertolotti are attempting to clone cDNAs from fibroblasts that extinguish the expression of liver-specific genes in hepatoma cells.

Maribeth Eiden has isolated a cDNA that causes chemically-transformed Baby Hamster Kidney (BHK) cells to revert to a normal phenotype. She will now sequence this "recessive oncogene" (tumor suppressor) and attempt to determine its mode of action.

K. and N. Okazaki and H. Okayama have developed a highly efficient method for transfecting schzosaccharomyces pombe. This strain of yeast responds to many mammalian regulatory sequences, and mutants can have their defects complemented by mammalian genes. Thus, schzosaccharomyces pombe appears to be a very powerful cloning host for characterizing proteins involved in the cell cycle, DNA repair, etc.

AIDS-Related research

M. Eiden and C. Wilson have succeeded in generating two gibbon ape leukemia and an HTLV1 recombinant viruses. These recombinant viruses are capable of infecting all cells infectable by their pathogenic wild type and counterparts, but they cannot replicate in infected cells. Therefore, that can safely and conveniently be used to study interactions between viruses and their receptors and to look for drugs or antibodies that interfere with viral binding to cells. In addition, the recombinant viruses provide an efficient means of introducing cloned genes into infectable cells.

Significance to Biomedical Research: The development and improvement of gene cloning techniques should greatly facilitate isolation and characterization of mammalian genes involved in various fundamental cell functions. Studies of genes involved in oncogenesis, cell growth, and differentiation are essential for understanding the mechanisms of these cell functions, and, ultimately, the pathogenesis of various diseases caused by disorders of these functions. Isolation of enzymes involved in repairing human DNA, for example, is directly related to discovering the genetic basis of the various forms of xeroderma pigmentosum.

Proposed Course of Project: The yeast expression cloning vector system will be further improved. The yeast vector system will be used for cloning DNA repair genes, genes controlling mitosis and meiosis, and genes for factors regulating tissue-specific gene expression. The gene that induces reversion will be recovered from the flat revertant of NMU-transformed cells, its gene and protein structure, its expression, and the function of the coded protein will be examined. The isolated TGF-beta insensitive mutants will be biochemically and genetically characterized, and will be used for complementation cloning of the genes that comprise the signal transduction pathways.

Publications:

Chen C, Okayama H. High-efficiency transformation of mammalian cells by plasmid DNA. *Mol Cell Biol* 1987;7:2745-52.

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Iacangelo A, Okayama H, Eiden LE. Primary structure of rat chromogranin A and distribution of its mRNA. *FEBS Lett* 1988;227:115-21.

Chen C, Okayama H. Calcium phosphate-mediated transfection: a highly efficient system for transforming cells with plasmid DNA. *BioTechniques* 1988;6:632-8.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02386-02 LCB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropeptide Secretion, Synthesis and Action in Neural, Endocrine and Immune Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Lee E. Eiden Chief, Unit on Molecular and Cellular Neurobiology, LCB, NIMH

See Attached

COOPERATING UNITS (if any)

U-44 INSERM, Centre de Neurochimie du CNRS, Strasbourg, France; VA Hospital, Washington, DC; LDN, NICHD; USUHS; Dept. Pathology, U Penn Med Ctr

LAB/BRANCH

Laboratory of Cell Biology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

6

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are examining the molecular mechanisms of neuropeptide secretion, neuropeptide expression and biosynthesis and neuropeptide interactions with their receptors in the developing and mature neuroendocrine system. We are attempting to understand the structural features of peptides and proteins that confer molecular specificity on these three processes. We hope to characterize and develop pharmacological agents that mimic this specificity.

Other Professional Personnel Engaged on Project:

D. Agoston	Guest Worker	LCB, NIMH
D. Brenneman	Pharmacologist	LDN, NICHD
M. Brownstein	Chief	LCB, NIMH
J. Dave	Visiting Scientist	NIAAA
J. Disbrow	Guest Worker	LCB, NIMH
R. Eskay	Chief, Section on Neurochem.	NIAAA
R. Fischer-Colbrie	Guest Researcher	NIMH
M. Grino	Guest Researcher	LCB, NIMH
C.-M. Hsu	Biologist	LCB, NIMH
A. Iacangelo	Microbiologist	LCB, NIMH
D. Lewis	Staff Fellow	LNP, NINCDS
J.-M. Muller	Guest Researcher	LCB, NIMH
H. Okayama	Visiting Scientist	LCB, NIMH
D. Perrin	Guest Researcher	LCB, NIMH
R. Pruss	Research Scientist	Merrell-Dow Research Institute
D. Rausch	Guest Researcher	LCB, NIMH
A. Rokaeus	Visiting Fellow	LCB, NIMH
R. Siegel	Asst. Professor	Dept. Pharmacol. Case-Western Res.
P. Smith	Asst. Professor	Dept. Anatomy, USUHS
K. Timmers	Assoc. Professor	Georgetown, D.C.V.A.
J. Waschek	Staff Fellow	LCB, NIMH

Project Description:

The Unit on Molecular and Cellular Neurobiology, as a part of the Laboratory of Cell Biology, investigates the molecular mechanisms regulating 1) expression and biosynthesis, 2) secretion, and 3) receptor-mediated action of neuropeptides in the diffuse neuroendocrine and immune systems, and *in vitro* models for these systems.

We have earlier defined stimulus-secretion-synthesis coupling as the second-messenger mediated phenomenon of simultaneous up-regulation of neuropeptide biosynthesis and secretion in response to receptor occupancy by physiological secretagogues. This second messenger is calcium in the case of acetylcholine-stimulated enkephalin secretion and synthesis in the adrenal medulla, and cyclic AMP in the case of corticotropin-releasing factor stimulation of pro-opiomelanocortin secretion and synthesis in the anterior pituitary gland. In the past year, these observations have been refined, especially using bovine chromaffin cells as a model of adrenomedullary peptide secretion and biosynthesis. The purpose of these further experiments has been 1) to identify intracellular proteins or factors that effect calcium-dependent secretion and biosynthesis and 2) to generalize the regulation of enkephalin biosynthesis and secretion to other neuropeptides contained in adrenal and other nervous tissue. Depolarization-stimulated enkephalin synthesis, but not secretion, is inhibited by phorbol myristate acetate. PMA probably modulates the activity of the same calcium-regulated third messenger which is stimulated by barium-dependent depolarization of chromaffin cells, since barium mimicks and replaces calcium in stimulating secretion, but not stimulation of biosynthesis and likewise, barium-stimulated biosynthesis is down-modulated by PMA, but barium-stimulated enkephalin secretion is not. Biosynthesis of various neuropeptides in chromaffin cells is differentially affected by elevation of second messengers: thus, galanin biosynthesis is stimulated most strongly by phorbol esters, substance P synthesis by cyclic AMP, VIP synthesis equally by cyclic AMP and phorbol esters, and neurotensin synthesis equally by stimulation of all second messenger systems

tested. Drs. Rokaeus, Eiden and Pruss have shown that galanin and enkephalin appear to be oppositely regulated by phorbol ester stimulation even though these peptides are contained within the same subpopulation of chromaffin cells. Despite their quite disparate individual regulation by protein kinase-linked second messenger systems, all these neuropeptides are released, and biosynthesized at an enhanced level, and in a calcium-dependent way, following depolarization with elevated potassium. Chang-Mei Hsu has obtained evidence that stimulus-secretion-synthesis coupling driven by cell depolarization occurs largely via calcium flux through L-type membrane channels, although mobilization of intracellular calcium, rather than calcium influx, may account for stimulation of peptide biosynthesis by some secretagogues, e.g. varatridine. In collaboration with Dr. Douglas Brenneman and his colleague Dr. George Foster, a similar regulation of enkephalin biosynthesis in neurons of the developing spinal cord *in vitro* to that in bovine chromaffin cells has been demonstrated. Spinal cord cells, which exhibit a high degree of spontaneous electrical activity, express both VIP and enkephalin peptides at levels comparable to depolarized (potassium-stimulated) chromaffin cells. Inhibition of spontaneous electrical activity by treatment with tetrodotoxin simultaneously (and reversibly) down-regulates enkephalin secretion and biosynthesis in these cells. Furthermore, treatment with phorbol esters decreases enkephalin levels about fifty percent, demonstrating that protein kinase C and calcium-dependent messenger systems interact in regulating enkephalin expression in a variety of neuroendocrine cells. Dr. Timmers has measured increased enkephalin mRNA in rat insulinoma cells after treatment with phorbol myristate acetate, suggesting that regulation of enkephalin expression by protein kinase C may be a cell-type-specific phenomenon. Dr. Fischer-Colbrie has examined enkephalin and neuropeptide Y mRNA expression as a function of the secretory state of the adrenal medulla *in vivo*, and has shown that biosynthesis of these neuropeptides is strongly regulated by activation of the splanchnic nerve to the adrenal medulla. Collectively, these data allow discernment of a very general mechanism of coupled neuropeptide secretion and synthesis regulation based on ambient intracellular activities of calcium, itself determined by the overall state of activation of the neuroendocrine cell. The role of second messengers like cyclic AMP and protein kinase C, may be to modulate enkephalin and VIP biosynthesis without affecting secretion, perhaps serving to establish and stabilize certain neuropeptide phenotypes in the developing neuroendocrine system.

The molecular basis of calcium-dependent regulation of secretion and biosynthesis will be studied in the coming year using a plaque assay system to measure peptide release from single chromaffin and neuroendocrine cells after intracellular injection and dye-marking of individual cells which has been developed by Drs. D. Perrin and P. Smith, coupled with single-cell analysis of neuropeptide mRNA abundance. If these methods are ultimately successful, the effects of intracellular injection of calcium-mobilizing and calcium-regulated intracellular messengers such as inositol trisphosphate, calmodulin and CAM kinase III, and substrate-based competitive inhibitors of CAM and other kinase systems, can be measured in neuroendocrine cells. At present, the intracellular actions of these messenger systems can be only inferred, and not observed directly, in bulk cell biochemistry experiments, since pharmacological inhibitors, and compounds which mobilize intracellular calcium such as inositol trisphosphate, do not cross cellular membranes and cannot be introduced into metabolically active cells by bulk transfer from liquid phase.

Chromogranin A is a ubiquitous neurosecretory protein contained within the adrenal medulla, pancreas, brain, retina, pituitary, enteric nervous system, peripheral sympathetic nervous system, a sub-population of large granular lymphocytes in rat, parathyroid, parafollicular C-cells of the thyroid, and in all species examined so far throughout the animal as well as protozoan kingdoms. Anna Iacangelo has previously cloned and

sequenced bovine chromogranin A, and demonstrated a structure suggestive of a prohormone precursor molecule. Based on a structural homology between a region of chromogranin A and pancreastatin, a biologically active peptide isolated from porcine pancreas by Tatemoto and co-workers, we proposed that chromogranin A may be the precursor for this peptide. Ms. Iacangelo has now cloned and sequenced porcine chromogranin A, and proved that it is the precursor for pancreastatin. She has also cloned and sequenced a rat chromogranin A cDNA, so that peptide, immunological, and nucleic acid reagents to probe the distribution, processing, regulation, and physiology of chromogranin A in this easily manipulated experimental animal can be conducted. So far, Drs. Fischer-Colbrie and Grino have demonstrated that the pituitary and adrenal reciprocally regulate chromogranin A mRNA expression, and that this regulation is mediated by glucocorticoids. Drs. Smith and Eiden have used chromogranin A antibodies to demonstrate with semi-quantitative histochemistry that the pituitary gland contains a far higher percentage of processed chromogranin A than the adrenal medulla. Dr. Rausch has studied the regulation of chromogranin A in PC-12 pheochromocytoma cells and demonstrated that chromogranin is a marker for two unique neuronal phenotypes generated by treatment with nerve growth factor and glucocorticoids, or nerve growth factor alone. Ms. Iacangelo has shown that chromogranin A mRNA is abundant in cell lines derived from the endocrine pancreas. Dr. Siegel has demonstrated using *in situ* hybridization histochemistry multiple sites of biosynthesis of chromogranin A including the basal ganglia of the brain. We are currently examining chromogranin A processing in various endocrine tissues, in order to identify the sites of biosynthesis of pancreastatin and other potential hormone products of chromogranin A. Employing this background and these reagents, we hope to demonstrate that chromogranin A is indeed the precursor of a biologically active hormone in the rat.

Dr. Rausch has attempted to extend her use of the PC-12 cell as a model for neuroendocrine lineage determination and mechanisms of neuronal differentiation by studying the ability of oncogenes of various classes to allow morphological and biochemical differentiation of PC-12 cells in a nerve-growth factor-independent manner. Dr. Rausch has identified a *src* (tyrosine kinase)-related, mRNA by Northern blot analysis which is distinguishable from both *n-src* and *c-src* oncogene messages in rat brain, which is significantly elevated in cerebellum and cerebral cortex at a time coincident with differentiating mitotic activity in these brain regions. Since *src* and *ras* oncogenes expressed transiently in PC-12 cells have been reported to cause neurite extension and changes in electrical excitability, Dr. Rausch constructed viral vectors to allow efficient and stable introduction of these oncogenes into PC-12 cells. She has found that *ras* expression does not cause stable differentiation of PC-12 cells, while *v-src* does: a temperature-sensitive *src* gene (*ts-src*) confers on stably infected PC-12 cells a (morphologically and electrophysiologically) differentiated phenotype which is temperature-sensitive and NGF-independent. The *v-src* tyrosine kinase appears to mimic the intracellular events which occur when NGF causes PC-12 cells to assume a neuronal phenotype. *Ts-src*-transformed PC-12 cells will be a suitable model system and source of starting material for biochemical and genetic analysis of the tyrosine kinase substrate, and the *v-src* endogenous *src* kinase analog, which effect neuronal differentiation in PC-12 cells.

Dr. Waschek has finished his study of the lineage-specific regulation of the VIP gene in neuroblastoma cells. The VIP gene contains multiple regulatory regions that function hierarchically to confer tissue-specific constitutive expression, second-messenger-dependent expression, or cell-type-conditional second-messenger-dependent expression in neuroblastoma cells. The element conferring tissue-specificity to VIP gene regulation lies between 3.6 and 5.2 kilobases upstream of the VIP promoter. Drs. Waschek and Agoston

now plan to extend these studies to examining the structural basis of tissue-specific calcium regulation of the VIP and other neuropeptide genes in primary neuroendocrine cells.

Drs. Waschek and Muller have compared VIP receptors, and their linkage to second messenger systems, in various subclones of neuroblastoma and in CD4-positive lymphoma cells. Both neuroblastoma and lymphoma cells possess high-affinity VIP receptors. Occupancy of the receptor by VIP results in rapid receptor down-regulation in both types of cells. VIP causes a rapid and robust (10-150 fold) increase in VIP in neuroblastoma cells, but little or no increase in cyclic AMP levels upon exposure to VIP in H9 lymphoma cells, although these cells respond to stimulation of adenylate cyclase by forskolin with a greater than 20-fold increase in cyclic AMP, demonstrating the patency of the lymphoma adenylate cyclase system. VIP receptor coupling thus differs dramatically between cells derived from the immune and central nervous systems, although the biochemical characteristics of the receptor are otherwise quite similar. This information may be relevant to the function of this receptor in the immune and central nervous systems.

A second antigen which is shared by the central nervous system and immunocytes is the CD4 antigen. This molecule plays a helper role in T-lymphocyte/monocyte interactions, and is the receptor for HIV, the etiological actor in acquired immunodeficiency syndrome. In the rat brain, this antigen has been shown to be strongly and locally up-regulated by brain injury. Mouse brain contains mRNA encoding CD4 and a shorter message potentially encoding a truncated version of the antigen. Mr. Disbrow has demonstrated that both full-length and truncated CD4 messages exist in human brain, and that the brain-specific truncated message is heterogeneously distributed in the central nervous system, while the full-length message is evenly distributed in all brain areas. Mr. Disbrow will study the distribution of CD4 antigen and its mRNA in human and primate brain tissue from individuals with head injury, neurodegenerative disease, and CNS infection, in order to determine how CD4 is regulated in the primate central nervous system, and compare it to CD4 regulation in the rat CNS.

Significance to Biomedical Research

Identification of the specific molecules that subserve neuropeptide secretion and synthesis, and the specific portions of each molecule involved in ligand-receptor interactions, will increase the likelihood of finding and designing specific pharmacological agents to mimic and to block these processes in the immune and nervous systems. These agents will allow further study of the structural features that impart specificity to the proteins involved in neuropeptide secretion and expression, the physiological consequences of blocking the secretion, synthesis or action of individual neuropeptides, and potentially specific blockade of pathogenic events mediated by proteins with similar (types of) structures.

Proposed Course of Project

The work described above will be followed to the endpoints of identifying pairs of molecules whose interaction within or on the cell is necessary for secretion, synthesis or action of specific neuropeptides or receptor antigens, and designing and testing peptide fragments and analogues that mimic or block those interactions. To this end, we are currently developing methods for studying the behavior of individual neuropeptide secreting and synthesizing cells and the effects of injection of purified proteins, peptides and other factors into them, as well as synthesizing fragments of identified receptors for which identified neuropeptides and lymphokines are ligands, and assaying their ability to inhibit ligand-stimulated cellular processes.

Publications:

O'Donohue, TL, Chronwall, BM, Pruss, RM, Mezey, E, Kiss, JZ, Eiden, LE, Massari, VJ, Tessel, RE, Pickel, VM, DiMaggio, DA, Hotchkiss, AJ, Crowley, WR, Zukowska-Grojec, Z. Neuropeptide Y and Peptide YY neuronal and endocrine systems. Peptides, in press, 1988.

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Beinfeld, MC, Brick, PL, Lowlett, AC, Holt, IL, Pruss, RM, Moskal, JR, Eiden, LE. The regulation of VIP synthesis in neuroblastoma and chromaffin cells. *Ann NY Acad Sci* 1987;**527**:68-76.

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Rieker, S, Fischer-Colbrie, R, Eiden, L, Winkler, H. Phylogenetic distribution of peptides related to chromogranins A and B. *J. Neurochem* 1988;**50**:1066-73.

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Siegel, RE, Iacangelo, A, Park, J, Eiden, LE. Chromogranin A biosynthetic cell populations in bovine endocrine and neuronal tissues: detection by *in situ* hybridization histochemistry. *Mol Endocrinol* 1988;**2**:368-74.

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Eiden, LE, Siegel, RE, Giraud, P, Brenneman, DE. Ontogeny of enkephalin- and VIP-containing neurons in dissociated cultures of embryonic mouse spinal cord and dorsal root ganglia. *Dev Brain Res*, in press, 1988.

Rausch, DM, Iacangelo, AL, Eiden, LE. Glucocorticoid- and nerve growth factor-induced changes in chromogranin A expression define two different neuronal phenotypes in PC12 cells. *Mol Endocrinol*, in press, 1988.

Eiden, LE. The enkephalin-containing cell: strategies for polypeptide synthesis and secretion throughout the neuroendocrine system. *Cell Mol Neurobiol* 1988;**7**:339-52.

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PROJECT NUMBER

Z01 MH 02387-02 LCB

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Analysis of the CD4/HIV Ligand/Receptor Dyad

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Lee E. Eiden Chief, Unit on Molecular and Cellular Neurobiology, LCB, NIMH

See Attached

COOPERATING UNITS (If any)

Genelabs, Inc.; Frederick Cancer Research Facility; Litton Bionetics;
Delta Regional Primate Cntr.; Sidney-Farber Cancer Institute

LAB/BRANCH

Laboratory of Cell Biology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

6

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A semi-purified peptide mixture consisting in benzylated derivatives of the CD4-derived peptide TYICEVEDQKEE has been further characterized as an HIV anti-infective, anti-cytopathic and virustatic agent in vitro, at a nominal concentration if 18-32µM. Purified and fully characterized derivatives of TYICEVEDQKEE have been identified which are anti-infective and anti-cytopathic at 40-80µM, but are less potent virustatic agents. One if these has been synthesized in large-scale (10-20 gm), partially purified and characterized, and will be injected into three rhesus macaque monkeys inoculated with the simian immunodeficiency virus B670 as a preliminary test of its anti-viral efficacy and safety in primates in vivo.

Other Professional Personnel Engaged on Project:

J. Lifson (co-P.I.)	Head	Division of Immunology, Genelabs, Inc.
P. Padgett	Chemist	LCB, NIMH
P. Nara	Unit Chief	LTCB, NCI
N. Dunlop	Microbiologist	LTCB, NCI
D. Rausch	Staff Fellow	LCB, NIMH
K. Hwang	Head	Division of Medicinal Chemistry, Genelabs, Inc.
B. Fraser	Senior Scientist	Division of Biologics, FDA
L. Martin	Senior Scientist	Delta Regional Primate Center, Covington, LA
M. Murphey-Corb	Senior Scientist	Delta Regional Primate Center, Covington, LA
L. Scharer	Professor	Dept. Pathology, Med. & Dental Coll. NJ
L. Epstein	Professor	Dept. Neurosci., Med. & Dental Coll. NJ
V.S. Kalyanaraman	Scientist	Litton Bionetics, Rockville
D. Diamond	Post-doctoral Fellow	Sydney-Farber Cancer Research Inst., Boston, MA
N. Murray	Senior Staff Fellow	LNP, NIMH

Project Description:

The CD4 antigen is a receptor for the human immunodeficiency virus (HIV) in T-cell and monocyte/macrophage infection. CD4 normally subserves class-II-restricted T-cell helper function in the immune system. It is also present in the central nervous system, where its function is unknown. We investigated the structural requirements for the CD4/HIV interaction to understand the fine-structural basis for peptide ligand-receptor interactions: the structures of both the gp120 ligand and the CD4 receptor are known, and functional assays for the ligand-receptor interaction exist, as do antibodies against both ligand and receptor. We have synthesized 20-25 amino acid fragments of the CD4 molecule, and tested these as competitive inhibitors of HIV infection and fusion of HIV-positive cells with uninfected CD4+ cells. None of the purified fragments were active to inhibit fusion of HTLV-IIIB-infected H9 lymphoma cells with VB CD4 positive indicator cells. A side fraction of the synthesis of one of the peptides [CD4(76-94)] did inhibit fusion completely at 125-250 μ M. Partial purification by differential extraction increased the nominal activity to 50 μ M. Chemical derivatization of the inactive parent peptide yielded preparations with nominal anti-viral activity of 60-120 μ M (complete blockade of fusion at these concentrations). The original material synthesized is also active in a direct assay for viral infection of CEM cells at a nominal concentration of 100 μ M (IC₅₀ 10 μ M). We hypothesized that the active peptide was a side product of the original synthesis due to incomplete removal of a protecting group during HF cleavage of the peptide from the resin. Accordingly, inactive authentic CD4(76-94) was synthesized, purified, and reacted with alpha-bromo-toluene to afford random side-chain benzylation of cysteine, threonine, tyrosine and potentially glutamic and aspartic acid. The subsequent adduct was biologically active at 125 μ M. On-line synthesis of CD4(76-94) deletion and substitution peptides containing S-benzyl cysteine allowed definition of a core sequence TYICEVEDQKEE [CD4(83-94)] S-benzyl peptide mixture, which possessed most of the activity of the parent 19-mer peptide mixture. Substitution of methionine, phenylalanine, serine or alanine for cysteine afforded inactive peptides. Peptides with the same amino acid composition but altered sequence were inactive. Peptides of unrelated sequence rich in threonine or glutamine acid, synthesized and cleaved under identical conditions to the 12-mer S-benzyl peptide were inactive.

Benzylated derivatives of CD4(83-94) have been shown to inhibit infection of CD4-positive human lymphoid cells by several strains of HIV-1, and by HIV-2, and to block HIV-1- and SIV-induced fusion between CD4-positive lymphoid cells and T-lymphocytoid cells, primary peripheral blood mononuclear cells, and macrophage cell lines in vitro, at concentrations between 30 and 250 μM (Lifson et al., Science 241:712, 1988; Lifson and Eiden, unpublished; Nara, Lifson, Hwang, Fraser, Dunlop, Rausch and Eiden, unpublished). The most potent of the peptides is an as yet uncharacterized side product of the synthesis of S-benzyl CD4(83-94), which has been purified to constant specific activity and is active at a nominal concentration of $<30 \mu\text{M}$ to inhibit fusion, cellular infectivity and infection in vitro. The next most potent and efficacious of this peptide series is a structurally defined multiply-benzylated derivative of TYICEVEDQKEE, which completely inhibits HIV-induced cell fusion and HIV-1 infection in vitro at 60 μM of the purified, structurally characterized peptide.

Significance to Biomedical Research:

Defining the requirements for antireceptor binding and development of potent antireceptor compounds will in general lead to a new class of pharmacophores with potential therapeutic and biological usefulness, and in this particular case to the development of an antiviral agent with significant therapeutic potential in treatment of a viral disease currently epidemic in the US, Africa and Europe.

Proposed Course of Project:

The structurally defined CD4(83-94) peptide derivative described above represents the lead compound in an ongoing drug development effort which has as its goal obtaining pharmacokinetic, toxicity and efficacy data in rhesus macaque monkeys infected with SIV, to guide future clinical tests of the compound in HIV-infected individuals. We intend to purify the material we have designated peak 7 to obtain structural definition of it, since it possesses significant virustatic activity not present in the structurally defined lead compound. Finally, we intend to perform experiments designed to validate our original hypothesis, that a single continuous epitope of a protein receptor may act as an antireceptor agent if sterically constrained in the appropriate conformation. These will examination of include peptide binding to purified gp120 HIV envelope glycoprotein, further structure-activity studies with CD4(83-94), and attempts to define regions of the CD4 molecule important in MHC class-II-restricted immune function.

Publications:

Lifson, JD, Hwang, KM, Nara, PL, Fraser, B, Padgett, M, Dunlop, NM, Eiden, LE. Synthetic CD4 peptide derivatives that inhibit HIV infection and cytopathicity. Science 1988;241:712-16.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02396-02 LCB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanical, Thermal and Optical Signs of Excitation in the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ichiji Tasaki	Chief, Unit on Neurobiology	LCB, NIMH
Others:	Paul M. Byrne	Biomedical Eng. Technician	LCB, NIMH
	Michio Masumura	Visiting Fellow	LCB, NIMH
		(appt. terminated 12/87)	
	Nobuko Tasaki	Guest Researcher	LCB, NIMH

COOPERATING UNITS (if any)

LNN, NICHD; LNC, NINCDS

LAB/BRANCH

Laboratory of Cell Biology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

1.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the past year, further investigations were conducted into the process of water movement and of energy transduction in the nervous system by measuring mechanical and thermal changes during excitation and inhibition. Continuous efforts were made to improve the sensitivity and the time-resolution of the detectors (devised and fabricated in this laboratory) employed in these investigations. Attempts were also made at constructing new types of detectors for measuring rapid mechanical, thermal and optical changes in the nervous system.

Significant achievements made during the past year include (1) elucidation of the process of nerve excitation and conduction in the garfish olfactory nerve by measuring both swelling and heat production during excitation, (2) analysis of the secretory process in the guinea pig pituitary gland by taking mechanical changes in the gland as an index, (3) studies of the mechanism of synaptic transmission in the cervical sympathetic ganglion of the guinea pig, and (4) demonstration of heat production of an unusually great magnitude evoked in the orthodromically activated olfactory bulb (bullfrog) in association with triphasic mechanical changes (swelling-shrinkage-swelling). All these studies are either already completed or near completion. It was repeatedly demonstrated in these studies that the method of recording non-electrical signs of nerve excitation and of synaptic transmission and inhibition reveals important properties of the nervous system which are inaccessible to conventional electrophysiological methods.

Project Description

Objective: The objective of the present research is to elucidate the function of the nervous system by examining non-electrical manifestation of excitation processes. In recent years, the principal investigator, working in collaboration with Paul M. Byrne, succeeded in greatly improving the sensitivity and the time-resolution of the heat-sensors fabricated in the laboratory by using thin polyvinylidene fluoride film. By using these improved heat-sensors, we wish to analyze the process of energy metabolism in the nervous system. (Note that biochemical methods of measuring the rate of energy metabolism have only limited time resolution.) Analyses of rapid mechanical changes observed during excitation of peripheral nerve fibers have yielded significant information about water movements associated with the release of bound Ca^{2+} ions in the cortical layer of nerve fibers. We wish to apply this method of recording rapid mechanical changes to studies of the mechanism of synaptic transmission in the central nervous system.

Methods: The heat-sensors fabricated in this laboratory has a heat-sensitive area of 2mm x 3mm and is capable of recording a rise in temperature of the order of 10 microdegrees centigrade with a time-resolution of roughly 5 milliseconds. A new type of heat-sensor which is capable of detecting rapid temperature changes in the superficial layer of the cerebral cortex was constructed; at present, however, the time resolution of this type of heat-sensor is limited. Piezoelectric transducers purchased from Gulton Industries, New Jersey, were employed for detection of very small forces developed at the surface of the nerve fibers and tissues during excitation. An attempt is being made to construct an electromagnetic device for detection of rapid surface movements of the nervous tissues during excitation.

Major Findings:

(1) Elucidation of the Process of Excitation and Conduction in the Garfish Olfactory Nerve

The olfactory nerve of the garfish, *Lepisosteus*, is a compact bundle of a large number (approximately 10 million) of long non-myelinated nerve fibers. This nerve is well suited for study in the process of nerve excitation and conduction because the constituent fibers have highly uniform diameters (0.2 - 0.3 μm). In

this nerve, the volume ratio of the membrane material to the axoplasm is known to be more than 5000 times as great as in the squid giant axon (see D. M. Easton, Science, vol. 172, p. 954, 1971). By using isolated olfactory nerves, we have succeeded in determining the time course of the heat produced in association with a conducted impulse at room temperature (20 - 22°). We found that the heat production in this nerve proceeds extremely rapidly, rising to its peak within about 10 milliseconds. In fact, the observed heat signals were found to rise more rapidly than the propagated action potential. (Note that the onset of a propagated action potential is determined primarily by the cable property of the axon.) The temperature rise associated with a propagated impulse was of the order to 10 μ deg. At low temperature (about 10 °C), the time course of heat production was disphasic; the phase of positive heat production was followed by a phase of heat absorption (as has been shown previously by A. V. Hill et al). In the nerve kept at room temperature, the amount of heat absorbed by this nerve was found to be almost negligibly small as compared with the heat generated. The possible significance of this unexpected finding will be discussed in the forth-coming manuscript. We also found that a propagated impulse is accompanied by a rapid swelling of the nerve. Furthermore, we proved that the peak of swelling coincides fairly accurately with the peak of the action potential. This finding may be interpreted as indicating that movement of water molecules is involved in the process of action potential production.

(2) Analyses of Secretory Process in the Guinea Pig Pituitary Gland.

The pituitary gland of the guinea pig is only about 2 mm in its length. Unsuccessful attempts were made to record heat production by the gland induced in response to electric stimuli applied to the stalk: the records obtained were severely distorted by large shock artifacts. However, we found it possible to detect rapid mechanical changes of the gland evoked by stalk stimulation. In isolated pituitary gland at room temperature, a single brief shock was found to evoke a rapid swelling of the gland which lasts for 20 - 30 milliseconds. The rise in pressure associated with this swelling was of the order to a few dyn/dm². When the stalk was stimulated repetitively, a short period of swelling was followed by a huge shrinkage of the gland. After immersion of the gland in saline solution containing barium or tetraethylammonium salts, large shrinkage of the gland could be evoked in response to a single stimulating shock. These

findings are now being analyzed by comparing with the results of biochemical studies of the gland carried out in the Laboratory of Neurochemistry, NINCDS

(3) Studies of the Mechanism of Synaptic Transmission in the Superior Cervical Ganglion of the Guinea Pig.

Because of the relative simplicity of its structure, the superior cervical sympathetic ganglion has been studied previously by a number of electrophysiologists. It was found possible to detect both mechanical and thermal signals from the ganglion in response to brief electric stimuli delivered to the presynaptic nerve fibers. Hexamethonium, d-tubocurarine, high Mg^{2+} were effective in blocking these orthodromically elicited mechanical and thermal signals. Immersion in a medium containing high Ca^{2+} enhanced the amplitude of the signals. Repetitive stimulation of the preganglionic fibers was found to produce fusion and summation of the signals. After addition of Ba^{2+} to the medium a single brief stimulus produced a huge, long-lasting shrinkage of the ganglion associated with only small heat production. All these findings are considered to be quite consistent with the results of previous electrophysiological investigations. of the ganglion.

(4) Demonstration of Triphasic Mechanical Changes in the Bullfrog Olfactory Bulb Associated with Monophasic Heat Production.

We found that the bullfrog olfactory bulb responds to both orthodromic and antidromic volleys of impulses with rapid mechanical and thermal changes of unusually great magnitude. The significant result of this study is that the powerful inhibition of mitral and tufted cells (induced by the well-known dendrodendritic interaction involving granule cells) is associated with profound shrinkage of the cells. The time-course of the mechanical response was invariably triphasic: it consisted of a phase of swelling (reflecting action potential production in the secondary neurons) followed by a phase of shrinkage (corresponding to the period of membrane hyperpolarization) and finally by swelling (probably reflecting resumption of repetitive firing of action potentials in the bulb). We found also the activation of the bulb is accompanied by large positive heat production. Attempts are being made at elucidating the nature of the process of shrinkage (and of heat production) associated with synaptic inhibition in the olfactory bulb.

Significance to Biomedical Research:

Our knowledge of the function of the vertebrate central nervous system is at present quite limited. Studies of non-electrical manifestations of the process of excitation and inhibition in the brain are expected to lead us to a better understanding of the normal, as well as abnormal, function of the nervous system.

Proposed Course of Project

We have just started investigating properties of the olfactory bulb and telencephalon of the bullfrog by using our improved thermal and mechanical sensors. In a preliminary study, we found that both thermal and mechanical changes in the telencephalic hemisphere can be readily detected by the use of our sensors. We are planning to explore the possibility of recording non-electrical signs of excitation processes in the superficial layer of the rat cortex. In addition to an expansion of the field of our investigation, a task we have to undertake in the immediate future is to carefully analyze all of the photographic signal records accumulated during the past two years in preparation for publication of the findings.

Publications

Tasaki I, Byrne PM, Masumura M. Detection of thermal responses of the retina by use of polyvinylidene fluoride multilayer detector, Japan J. Physiol 1987;37:609-19.

Tasaki I. A macromolecular approach to excitation phenomena: mechanical and thermal changes in nerve. To be published in a book "Cases to Answer", Hillman H. Ling, G, ed. and in the Journal of Physiology, Chemical Physiology and Medical NMR, Pennsylvania.

Other Activities

(1) I. Tasaki participated in an International Workshop on Applications of Polyelectrolyte Gels" here at NIH, Jan 11 - 13, 1988.

(2) He participated in a mini-symposium on "New Theoretical Approaches to Membrane Excitability" held at the annual meeting of the Biophysical Society, March 2, 1988: The title of his 30 min. presentation; "A theory of Two Stable States of the Nerve Membrane.

(3) He has been chosen as a member of the delegates of American biophysicists and is scheduled to visit the Peoples' Republic of China, November 9 - December 1, 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00881-32·LCM
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Intermediary Energy Metabolism in Mammalian Brain		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	E. E. Kaufman	Research Chemist LCM, NIMH
Others:	T. Nelson	Medical Officer (Research) LCM, NIMH
COOPERATING UNITS (if any) Laboratory of Chemistry, NHLBI (Henry M. Fales)		
LAB/BRANCH Laboratory of Cerebral Metabolism		
SECTION Section on Developmental Neurochemistry		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.35	PROFESSIONAL: 0.20	OTHER: 0.15
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This report will describe recent findings on the mechanism of action and properties of a new mammalian <u>mitochondrial transhydrogenase</u> which catalyzes the transfer of hydrogen from a hydroxyacid to an oxoacid. Quantitative transfer of deuterium from the hydroxygl bearing carbon of a hydroxyacid to the α -carbon of α -ketoglutarate has now been demonstrated. Purification and preliminary determination of the molecular weight of this enzyme are being carried out.		

Project Description:Objectives:

The objectives of this study have been the isolation, characterization and purification of a mitochondrial hydroxyacid-oxoacid transhydrogenase found in brain, liver and kidney. The role of this transhydrogenase in the metabolism of such key metabolic intermediates as L- β -hydroxybutyrate, α -hydroxyglutarate, and γ -hydroxybutyrate is also of interest.

Methods:

The mitochondrial transhydrogenase described in this report has been purified using the following techniques: 1) differential centrifugation, 2) salt fractionation, and 3) column chromatography. The products of the reaction have been identified using gas-liquid chromatography either alone or in combination with mass spectroscopy, paper and thin layer chromatography. DL- γ -deutero- γ -hydroxybutyrate was prepared from succinic semialdehyde and NaBD₄. The product of this reaction DL- γ -deutero- γ -hydroxybutyrate was determined to be 80% deuterated. The product, α -hydroxyglutarate, which was formed when the DL- γ -hydroxybutyrate was incubated with α -ketoglutarate in the presence of partially purified transhydrogenase was approximately 40% deuterated. This was almost exactly the expected incorporation of deuterium since the deuterium on only one of the optical isomers of the DL-compound would be transferred to the α -ketoglutarate. This is also an indication that a chiral enzyme-bound intermediate is involved. Work on the purification and characterization of this enzyme is being completed.

This is the first report of (1) a mammalian mitochondrial transhydrogenase capable of transferring a hydrogen from a hydroxyacid to an oxoacid, and (2) a mammalian enzyme capable of catalyzing the oxidation of free L- β -hydroxybutyrate.

Significance to Biomedical Research and to the Program of the Institute:

A completely new enzyme which can catalyze the transfer of hydrogens from hydroxyacids to oxoacids has been isolated. The discovery of this enzyme may lead to new insights concerning the metabolism of certain hydroxyacids, in particular L- β -hydroxybutyrate and γ -hydroxybutyrate, as well as the metabolism of the Krebs cycle intermediate, α -ketoglutarate.

These findings also indicate a potentially new regulatory rule for α -ketoglutarate.

Publications:

Kaufman, EE. Dual pathways for the catabolism of γ -hydroxybutyrate: cytosolic and mitochondrial mechanisms. In: Kleinkauf, von Dohren, Jaenicke, eds. The roots of modern biochemistry: energetics of the cell. Berlin, New York: Walter De Gruyter and Company, 1988;867-878.

Kaufman, EE, Nelson, T, Miller, D., Stadlan, N. Oxidation of γ -hydroxybutyrate to succinic semialdehyde by a mitochondrial pyridine nucleotide-independent enzyme. J Neurochem, 1988, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00882-21 LCM
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies on Regional Cerebral Circulation and Metabolism		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	L. Sokoloff	Chief LCM, NIMH
Others:	C. Kennedy	Guest Researcher LCM, NIMH
	T. Nelson	Medical Officer (Research) LCM, NIMH
	C. B. Smith	Research Chemist LCM, NIMH
	G. A. Dienel	Senior Staff Fellow LCM, NIMH
	N. Cruz	Biologist LCM, NIMH
	N. Eng	Chemist LCM, NIMH
COOPERATING UNITS (if any) Theoretical Statistics & Mathematics Branch, NIMH (C.S. Patlak & K.D. Pattigrew); NINCDS, NIH (I. Kopin & L. Porrino)		
LAB/BRANCH Laboratory of Cerebral Metabolism		
SECTION Section on Developmental Neurochemistry		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
9.50	6.00	3.50
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The deoxyglucose method for the quantitative determination of rates of local <u>glucose consumption</u> in the discrete functional and structural components of the <u>brain</u> of conscious or anesthetized laboratory animals was developed in this laboratory over 10 years ago. In this method [¹⁴ C]deoxyglucose is employed as a tracer for glucose flux through the hexokinase step; the product, [¹⁴ C]deoxyglucose-6-phosphate, is measured by quantitative autoradiography. The method continues to be used to study alterations in local <u>energy metabolism</u> in a variety of physiological, pharmacological and a limited number of pathological states. Its suitability to a wider range of pathologic conditions is being extended and special time constraints which may be present in the method's adaptation for use in human subjects with [¹⁸ C]fluorodeoxyglucose and PET have been examined.		

OTHER INVESTIGATORS (CONTINUED)

Kathleen Schmidt	Computer Systems Analyst	LCM, NIMH
John Viola	Guest Researcher (Howard Hughes Scholar)	LCM, NIMH
Giovanni Lucignani	Guest Researcher	LCM, NIMH
Kentaro Mori	Visiting Fellow	LCM, NIMH
Quang Vo	Computer Programmer	LCM, NIMH
Ernesta Palombo	Visiting Fellow	LCM, NIMH
Hajime Nakanishi	Visiting Fellow	LCM, NIMH

Project Description:

The deoxyglucose method, both in its original form and in its adaptation for use in human subjects, has been widely used by investigators throughout the world for over a decade. It has also been employed by members of this laboratory in the study of a variety of physiological conditions as reported previously and as given below. In the interests of extending the method's general applicability, the laboratory has refined the model on which the method is based. This revision takes into account new information on the intracellular sites of phosphatase activity, and thereby defines more accurately the late time course when radioactive label is lost from the tissue. The new model provides the basis for understanding the time limits after administration of the labeled deoxyglucose during which valid measurements can be made, and to correct for processes that become significant with the long scan times required when PET is employed. Also many of the experiments planned over a year ago to permit the deoxyglucose method to be extended for use in a wide range of pathophysiologic states have been successfully accomplished, and work done in response to criticisms by others has been concluded. These diverse studies related to the deoxyglucose method are separately described below.

I. APPLICATIONS OF THE DEOXYGLUCOSE METHOD

A. **Primate Model of Parkinsons Disease.** Dr. Linda Porrino (NINCDS), Dr. Ernesta Palombo and John Viola (HHMI), in collaboration with Drs. Irwin Kopin, Krystof Bankiewicz and Robert Plunkett of NINCDS, have extended their studies with the deoxyglucose method applied to the Parkinson's disease in a primate model of hemiparkinsonism induced by unilateral intra-carotid injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

Objectives:

- 1) To measure glucose utilization in the neural pathways involved in the production of abnormal motor function in hemiparkinsonian monkeys and to identify those circuits involved in amelioration of the syndrome by apomorphine.

- 2) To map the neural circuits in hemiparkinsonian monkeys that subserve the therapeutic improvements that accompany dopaminergic implants into the caudate.

Major Findings:

Previous work has shown that MPTP-induced hemiparkinsonian monkeys is accompanied by selective metabolic changes in the striatum, external globus pallidus, and subthalamic nucleus. L-DOPA treatment reverses the changes in glucose utilization in the subthalamus and striatum, but not the pallidum. In contrast, apomorphine administration reduced metabolic increases in the external globus pallidus and induced metabolic reduction in the subthalamus. A patchy pattern of increases in glucose utilization was observed in the striatum ipsilateral to the side of the lesion.

A recent approach to the treatment of Parkinson's disease has been the use of dopamine-producing tissue implanted into the brains of patients with the disease. Drs. Porrino, Palombo and Viola have used the deoxyglucose method to assess functional recovery in hemiparkinsonian monkeys with such implants. The pattern of glucose utilization seen in monkeys with adrenal implants in which no recovery of voluntary movement was observed was identical to that seen in untreated hemiparkinsonian monkeys. In contrast, following fetal mesencephalic tissue implants, recovery of motor function was evident along with reversal of the glucose utilization changes associated with hemiparkinsonism.

B. Rapid-Eye-Movement Sleep in the Fetus. In a continuation of the Laboratory's long standing interest in the cerebral metabolism of sleep, a collaboration was undertaken with Dr. Robert Abrams, of the University of Florida in Gainesville. During fetal life rapid eye movement (REM) sleep occupies nearly 50% of total sleep time, and the duration of REM periods may be as long as 20 minutes. Dr. Abrams' expertise in studies of the sheep fetus made possible a collaboration in which the deoxyglucose method could be applied to learn of local cerebral glucose utilization during REM sleep. In a very unique study we have learned that REM periods are accompanied by marked increases in the metabolic rate of the brain. These increases appear not to be localized but involve virtually all brain regions. An account of this study has recently been published (Abrams, RM, Hutchison, AA, Jay, TM, Sokoloff, L, and Kennedy, C. Developmental Brain Research 1988;40:65-70.

C. Effects of Phencyclidine on Local Cerebral Glucose Utilization. A project is being carried out in collaboration with Dr. Kenneth Keilar of the Department of Pharmacology at Georgetown University School of Medicine. The experimental work, in which PCP was employed in a wide dose range in the rat, has now been completed. The study is similar in many respects to those reported for others who have used the deoxyglucose method to evaluate this agent, but our study will provide new information on its effect at relatively low doses. The data analysis is in progress.

II. EXTENSION OF THE DEOXYGLUCOSE MODEL TO A 5-PARAMETER MODEL

New information about the fate of [^{14}C]deoxyglucose once it has entered brain cells was provided by Fishman and Karnovsky (J. Neurochem. 46:371, 1986). These investigators found evidence that the hydrolysis of deoxyglucose-6-phosphate is rate-limited by its diffusion across the endoplasmic reticular membrane to the site where glucose-6-phosphatase resides. The original model on which the deoxyglucose method was based was, therefore, revised to include this new information. A new equation was formulated which contained five rate constants, two additional rate constants being introduced to account for the constraints of the revised model. Drs. Giovanni Lucignani, Kentaro Mori, Therese Jay, Ernesta Palombo, Thomas Nelson and Mrs. Kathleen Schmidt have completed the experimental work. The new equation was fit to the measured data by a non-linear, least-squares routine to obtain the best rate constants. The data were also analyzed by the graphical evaluation technique which provides an estimate of the time when loss of product begins to take place. The manuscript on this work is virtually completed.

III. ADAPTATION OF THE DEOXYGLUCOSE METHOD FOR USE IN PATHOPHYSIOLOGICAL CONDITIONS

The deoxyglucose method for the quantitative measurement of local cerebral glucose utilization in brain was originally designed for use in laboratory animals under physiologic conditions. When physiologic conditions are exceeded, as in ischemia, prolonged seizures, cerebral edema and severe hypoglycemia, the value for the lumped constant of the operational equation of the method is altered. In order to apply the deoxyglucose method to such pathophysiologic conditions experiments are being designed which will allow the lumped constant to be determined in the same experiment in which local cerebral glucose utilization is measured. Through the use of [^3H]methylglucose it is now possible to measure local glucose concentration in the brain. This, in turn, makes possible an estimate of the ratio of the distribution volumes and, therefore, of the lumped constant itself.

Objectives:

To make possible the determination of the local lumped constant in the brains of animals under pathophysiologic conditions. Thus, the determination of glucose utilization in all local subdivisions of brain could be reliably measured in pathophysiologic states.

Major Findings:

1. Methylglucose can be used to estimate local glucose concentration in brain only if it is uncontaminated by products of its metabolism. Recent reports of methylglucose phosphorylation by yeast and heart hexokinase made it necessary to assay brain for any possible products of its metabolism after methylglucose had been introduced into the circulation.

T. Jay, N. Cruz, and G. Dienel were unable to find significant quantities of any metabolic product of methylglucose in plasma or brain over a wide range of plasma concentrations. Small quantities of acidic derivatives were found, however, in liver and heart. These experiments served to validate the use of methylglucose as a means of determining local glucose concentration in brain. This work was completed last year and reported in abstract form (T. Jay, N. Cruz, T. Nelson, L. Sokoloff and G. Dienel, *Journal of Cerebral Blood Flow and Metabolism*, 7:S491, Suppl. 1, 1987). The full manuscript reporting the complete work is now in preparation.

2. Autoradiographs made with [^{14}C]methylglucose at high (500 mg/dl) and low (40 mg/dl) glucose concentrations in plasma indicate that the glucose concentration, and therefore the lumped constant, does not vary throughout brain under these conditions.
3. The complex experiments in which concentrations of glucose, deoxyglucose and methylglucose were determined in brain over a wide range of plasma glucose concentrations have now been completed by K. Mori, N. Cruz, and G. Dienel, and the family of curves of their distribution volumes have been plotted. The results provide confirmation of the values for the lumped constant in the rat (as originally determined from a measurement of the ratio of extraction ratios in the blood/plasma of [^{14}C]deoxyglucose and glucose during a steady state) for plasma glucose levels between 80-500 mg/dl. The plots will provide the basis for the calculation of the local lumped constant in pathophysiologic states in which local glucose concentrations are estimated from autoradiographs of brain sections made with [^{14}C]methylglucose.
4. At plasma glucose levels below 80 mg/dl a discrepancy was found between values for the lumped constant determined from a direct assay of plasma and tissue concentrations of deoxyglucose and glucose. This appears to be the result of the conversion of a significant fraction of deoxyglucose-6-phosphate in the cells to unidentified metabolic products in hypoglycemia. Cruz and Dienel are currently developing a new tissue extraction procedure that will preserve these metabolites. The distribution ratios for deoxyglucose and glucose will then have to be corrected from a knowledge of the concentrations of these metabolites over a range of hypoglycemia.
5. A double label autoradiographic procedure has been developed to make possible the measurement of local [^{14}C]deoxyglucose and [^3H]methylglucose concentrations in the same section of brain. This involves the use of mylar film which blocks all radiation from tritium with minimal reduction of that from ^{14}C . The mylar will be used to cover the sections during a second exposure of both brain sections and standards. Thereby the separate contribution of each isotope to the optical densities of the autoradiographs will be determined. The calibration of tritium standards for their equivalent brain concentration of ^3H is presently in progress.

IV. WORK IN RESPONSE TO PAPERS CRITICAL OF THE DEOXYGLUCOSE METHOD

The issues raised by a few vocal critics in the past have now been silenced as a result of a laboratory effort spanning several years. Two accounts from this laboratory remain to appear as full publications: one is that recently submitted to the Journal of Biological Chemistry by Dr. Gerald Dienel et al. He and coworkers showed that methodological deficiencies in isolation procedures and incomplete recovery of labeled metabolites of glucose and deoxyglucose were sufficient to explain the results of Huang and Veech. These authors had claimed to provide evidence for previously unrecognized high levels of glucose-6-phosphatase activity in brain. The other work by Mori et al. shows a negligible role of glucose-6-phosphatase in affecting calculated results in the deoxyglucose method. Loss of label was examined by the model independent, multiple time/graphic approach of Patlak et al. (J. Cereb. Blood Flow Met. 3:1, 1983). The manuscript describing these studies is in preparation.

Significance to Biomedical Research and to the Program of the Institute:

The deoxyglucose method has made it possible for the first time to measure the rates of glucose utilization simultaneously in all functional and structural components of the central nervous system of conscious, behaving animals and now also in man. Because the method was developed in our Laboratory, it has been our responsibility to survey its applicability to the various types of conditions in which it might be useful. The program has, therefore, been somewhat heterogeneous covering a wide range of physiological, pharmacological, pathological, and altered behavioral states. The method and its wide-ranging usefulness have now been more or less established, and it is used extensively throughout the world in neuroanatomical, neurophysiological, neuropharmacological, psychiatric, neurological, and neurosurgical research. Its wide acceptance is directly related to the results of studies in this project.

In the study of Parkinsonism the data demonstrate differential effects of dopaminergic agents used for reversal of the signs of the disorder, suggesting that different neuronal circuits may be capable of mediating improvements in voluntary motor function. In addition, the selectivity of these agents may provide the basis for identifying the functional roles of the separate outflow pathways of the striatum. Additionally, the therapeutic potential of fetal implants in Parkinson's disease is underscored, in that fetal implants not only normalized motor performance but restored symmetry in cerebral metabolic rates which was not seen following adrenal implants.

Additional knowledge has been gained on the metabolic characteristics of REM sleep. Inasmuch as the electrophysiologic manifestations of REM sleep during fetal life are virtually identical to those in the postnatal period the metabolic findings in the fetus are relevant to REM sleep at other age periods.

Proposed Course:

Applications of the deoxyglucose method to problems in all the disciplines mentioned above will be continued. A project has been initiated and will be continued to adapt the method for use in neuropathological conditions such as stroke, status epilepticus, etc. Efforts will be made to improve the quantitative resolution of the method to the single cell and subcellular levels. Immunocytochemical techniques will be introduced with the aid of Dr. Bernard Driscoll to correlate local cerebral rates of glucose utilization with local levels of neuropeptides and the host of putative neurotransmitters and neuromodulators. A cell culture facility has been established in the Laboratory by Dr. B. Driscoll to allow studies of cellular mechanisms of carbohydrate transport across cell membranes which are necessary to define the rates of glucose utilization in neuronal and glial cellular components of the cerebral tissue.

Metabolic mapping studies will be continued in order to investigate the effects of selective D₁ and D₂ agonists in the hemiparkinsonian monkey. The local metabolic effects of implants of fetal brain will be extended.

New information is being provided on somatomotor and somatosensory localization in cerebral cortex as well as a delineation of the extent of the inferior parietal lobule which subserves eye-hand movement.

Publications:

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Nelson T, Dienel GA, Mori K, Cruz NF, Sokoloff L. Deoxyglucose-6-phosphate stability in vivo and the deoxyglucose method: response to comments of Hawkins and Miller, J Neurochem 1987;49:1949-1960.

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Sokoloff L. Basic principles in imaging of regional cerebral metabolic rates with radioisotopes. (Proceedings of the NATO ASI Meeting in L'Aquila, Italy, June, 1986), in press.

Sokoloff L. Circulation and energy metabolism of the brain. Basic Neurochem 4th Ed. Raven Press, in press.

Sokoloff L, Kennedy C, Smith CB. The [¹⁴C]deoxyglucose method for measurement of local cerebral glucose utilization. Neuromethods Vol. 15. Humana Press, in press.

Sokoloff L. Metabolic probes for localization of functional activity in the central nervous system. Int J Neurol, in press.

Palombo E, Porrino LJ, Bankiewicz KS, Crane AM, Kopin IJ, Sokoloff L. Administration of MPTP acutely increases glucose utilization in the substantia nigra of primates. Brain Res, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00887-11 LCM
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Extended Visual System of the Macaque Monkey		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: C. Kennedy Others: L. Sokoloff M. Mishkin	Guest Researcher Chief Chief	LCM, NIMH LCM, NIMH LN, NIMH
COOPERATING UNITS (if any) Laboratory of Neuropsychology, NIMH (M. Mishkin)		
LAB/BRANCH Laboratory of Cerebral Metabolism		
SECTION Section on Developmental Neurochemistry		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 1.25	OTHER: 0.75
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p> The <u>deoxyglucose method</u> has been applied to monkeys which responded to visual cues by lever pressing with one hand. The project began with its focus on mapping the <u>visually responsive cortical areas</u>, but has now been extended to a study of the <u>sensory motor pathways</u>. An asymmetrical pattern of glucose utilization is found in a wide expanse of cortical and subcortical structures. A quantitative analysis of this asymmetry provides detailed information about the locus and amount of <u>neuronal activity</u> induced by the visually cued motor activity. The demands of other projects in the laboratory have delayed progress on this project this year. </p>		

Project Description:

Objective:

To map all regions of monkey brain which are involved in the performance of a visually cued task involving motor activity in one arm/hand

Methods Employed:

Normal monkeys engaged in unimanual lever pressing in response to visual cues were studied with the deoxyglucose method. To the extent that the induced neuronal activity results in asymmetry in local metabolic rates, an analysis of autoradiographs for right-left differences provides information on the locus and extent of participating regions.

Major Findings:

The analysis to date has indicated that the metabolically activated regions are widely distributed in both cortical and subcortical structures. Most prominently involved cortical areas are limited parts of the primary motor (M₁), primary sensory (S₁), the secondary sensory (S₂) cortices and supplemental motor areas. Activation of a discrete part of the superior parietal lobule, part of area 7, appeared to provide new information on this light-sensitive region of cortex. Deep structures unilaterally affected were parts of the thalamus (VPM) and (VPL), subthalamus, substantia nigra and red nucleus. Part of crus II of the cerebellum was found to be elevated in its metabolic rate on the side ipsilateral to the moving arm.

The priorities of other projects has delayed the completion of this project.

Significance to Biomedical Research and to Program of the Institute:

New information is being provided on somatomotor and somatosensory localization in cerebral cortex as well as a delineation of the part of the inferior parietal lobule which subserves visually guided hand movement in brain.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00889-09 LCM																																
PERIOD COVERED October 1, 1987 to September 30, 1988																																		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) A Method for the Determination of Local Rates of Protein Synthesis in Brain																																		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI:</td> <td style="width: 40%;">C. B. Smith</td> <td style="width: 30%;">Research Chemist</td> <td style="width: 10%;">LCM, NIMH</td> </tr> <tr> <td>Others:</td> <td>L. Sokoloff</td> <td>Chief</td> <td>LCM, NIMH</td> </tr> <tr> <td></td> <td>C. Eintrei</td> <td>Visiting Fellow</td> <td>LCM, NIMH</td> </tr> <tr> <td></td> <td>C. Kennedy</td> <td>Guest Researcher</td> <td>LCM, NIMH</td> </tr> <tr> <td></td> <td>G. Deibler</td> <td>Research Chemist</td> <td>LCM, NIMH</td> </tr> <tr> <td></td> <td>K. Schmidt</td> <td>Computer Systems Analyst</td> <td>LCM, NIMH</td> </tr> <tr> <td></td> <td>M. Mishkin</td> <td>Chief</td> <td>LN, NIMH</td> </tr> <tr> <td></td> <td>R. Nakamura</td> <td>Guest Researcher</td> <td>LN, NIMH</td> </tr> </table>			PI:	C. B. Smith	Research Chemist	LCM, NIMH	Others:	L. Sokoloff	Chief	LCM, NIMH		C. Eintrei	Visiting Fellow	LCM, NIMH		C. Kennedy	Guest Researcher	LCM, NIMH		G. Deibler	Research Chemist	LCM, NIMH		K. Schmidt	Computer Systems Analyst	LCM, NIMH		M. Mishkin	Chief	LN, NIMH		R. Nakamura	Guest Researcher	LN, NIMH
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COOPERATING UNITS (if any) Dept. Neurosurgery, U. Texas (M. Kadekaro); Dept. Obstet. & Gynecol., U. Fla. (R. Abrams); Dept. Neurosurgery, SUNY (D. Dow-Edwards); Dept. Neurol., U. Mass. Med. Sch. (W.J. Schwartz, T. Scammell); Dept. Anatomy, CUNY Med. Sch. (A. Yu); Dept. Psychiatry, UCSD (J.C. Gillin)																																		
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INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892																																		
TOTAL MAN-YEARS: <div style="text-align: center;">2.9</div>	PROFESSIONAL: <div style="text-align: center;">2.2</div>	OTHER: <div style="text-align: center;">0.7</div>																																
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																																		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>A method has been developed for the estimation of local rates of <u>protein synthesis</u> in brain <u>in vivo</u> by <u>quantitative autoradiography</u>. The method is based on the use of L-[1-¹⁴C]<u>leucine</u> as a tracer for the incorporation of leucine into protein. A <u>four-compartment model</u> for the behavior of leucine in brain has been analyzed, and an equation has been derived that defines the rate of amino acid incorporation into protein in terms of the time course of plasma-specific activity, final tissue concentration of ¹⁴C, and λ, the ratio of the distribution volumes for the labeled and unlabeled leucine. A value of λ for the brain as a whole has been experimentally determined. A number of studies have been carried out in order to ascertain the potential usefulness of the method. These studies include: 1) normal <u>development</u>; 2) <u>slow wave sleep</u>; 3) <u>barbiturate anesthesia</u>; 4) <u>ketamine anesthesia</u>; 5) <u>electrical stimulation of the sciatic nerve</u>; 6) <u>hypothyroidism</u>; 7) <u>circadian rhythms</u>; and 8) effects of <u>testosterone</u> on <u>regenerating neurones</u>.</p>																																		

Project Description:Major Objectives:

The overall objectives of this research project are:

1. To develop and validate a method for the measurement of local rates of protein synthesis in brain.
2. To test the usefulness of the method for the study of neurobiological problems.

A method is being developed for the estimation of local rates of protein synthesis in brain in vivo. This method is similar to the [^{14}C]deoxyglucose method in that it is based on enzyme kinetic principles, as applied to the measurement of reaction rates in vivo with labeled tracers as substrates and quantitative autoradiography. The two requirements for this type of method are: 1) it must be possible to determine the amount of labeled product formed in the tissue during the experimental interval, and 2) it must be possible to determine the integrated specific activity of the precursor at the site of the reaction over the entire time course of the interval in terms of measurable variables. In order to satisfy the first requirement, L-[1- ^{14}C]leucine has been chosen as the radiolabeled tracer. The only metabolic pathway for leucine, apart from protein synthesis, entails a transamination followed by a very rapid decarboxylation. Therefore, in the metabolism of [1- ^{14}C]leucine the label is transiently transferred to α -ketoisocaproic acid and ultimately to $^{14}\text{CO}_2$ which is then diluted in the large CO_2 pool in brain produced by carbohydrate metabolism and rapidly lost. There are, therefore, no residual radioactive products other than labeled protein. In order to meet the second requirement we have attempted to develop a kinetic model based on the known biochemical behavior of leucine in the brain. We have analyzed numerous kinetic models for the behavior of leucine in brain and found that, regardless of their complexity in terms of number of tissue compartments and their interrelatedness, the operational equation for the determination of the local rates of protein synthesis always has the same general form. The numerator is composed of the total concentration of radioactivity in the tissue at the end of the experimental period, minus term(s) for the amount of radioactivity in each of the free leucine pools in the tissue. The denominator is composed of the integrated specific activity in the plasma, minus term(s) for the lag between the precursor pool in the tissue and the plasma. This "lag-corrected" integrated specific activity is multiplied by λ , the ratio of the distribution volumes of the labeled and unlabeled leucine in the leucyl-tRNA pool. The terms in the numerator for the free leucine pools can be eliminated by fixing and washing tissue sections. The expressions for the lag in the denominator can be minimized by the administration of a pulse of [^{14}C]leucine and allowing 60 minutes for clearance of free [^{14}C]leucine from the plasma and the brain. Sixty minutes is estimated to be more than 10 half-lives of the precursor pool in white matter, the tissue with the largest half-life. The value of λ must be determined in a separate series of experiments.

The specific aims pursued during this fiscal year were:

1. To complete experiments designed to determine λ , the equilibrium distribution ratio for labeled leucine in the leucyl-tRNA pool divided by the equilibrium distribution ratio for unlabeled leucine in the leucyl-tRNA pool.
2. To continue studies of the applications of the [^{14}C]leucine method to various neurobiological problems.

Methods Employed:

1. The determination of λ .

The experiment consists of the determination of the ratios of endogenous unlabeled leucyl-tRNA to plasma leucine and [^3H]leucyl-tRNA to plasma [^3H]leucine in a rat in a steady state for both labeled and unlabeled leucine. A programmed intravenous infusion schedule for [^3H]leucine was designed that produces and maintains a constant concentration of [^3H]leucine in the arterial plasma. This input function was obtained from a LaPlace transform of the relationship between a pulse input of [^3H]leucine and the multiexponential output in the arterial plasma. The controlled infusion is achieved by means of a programmable infusion pump. Rats are maintained in a steady state for both labeled and unlabeled leucine for at least 30 minutes at which time they are decapitated and the brains and livers rapidly removed. Brain and liver tRNA-amino acids are separated and purified by differential centrifugation and acid and phenol extraction techniques. Purified tRNA-amino acid is deacylated at pH 10 and the amino acids are separated from the freed tRNA by ethanol precipitation. The specific activity of the leucine in this amino acid fraction and the specific activity of the leucine in the plasma samples are determined by either of two methods: 1) derivatization with [^{14}C]dansyl chloride, separation of [^{14}C]dansyl-[^3H]leucine by TLC and HPLC and double label liquid scintillation counting; or 2) ion-exchange chromatography, post-column derivatization with ortho-phthalaldehyde (OPA), and fluorescent detection. Gladys Deibler has developed the second method for the determination of picomolar levels of amino acids with the Beckman 7300 High Performance Amino Acid Analyzer and post-column derivatization with OPA. Amino acids, separated by means of standard ion exchange chromatographic techniques, are reacted with OPA and the reaction products are detected and quantitated with a fluorometric detector. After detection, column eluates are collected and counted in order to determine specific activity. With this system as little as 1 pmole of leucine could be detected and quantitation was reliable between 10 and 50 pmoles.

Major Findings:1. The determination of the value of λ for the brain and liver.

Values of λ have been obtained in a series of adult, male rats with both analytical techniques. In the brain, values of λ were between 0.54 and 0.62 with a mean value of 0.58. In the liver, values of λ were between 0.39 and 0.59 with a mean value of 0.47. The fact that values obtained were similar in both 30 and 60 minute experiments shows that a steady state had been reached by 30 minutes.

2. Studies of neurobiological problems.

In order to examine the potential usefulness of the leucine method, studies of neurobiological problems have been pursued with the assumptions that the value of λ is 1.0 and that it does not change with the experimental conditions. We have determined local rates of protein synthesis in normal, conscious, adult, male rats. Average values for the brain as a whole are 2-3 nmoles/g/min. Values are highest (8-14 nmoles/g/min) in hypothalamic nuclei known to produce and export peptide hormones and lowest (1-2 nmoles/g/min) in regions of white matter. Regions of cortex and subcortical grey matter structures have values between 2 and 7 nmoles/g/min.

Dr. C. Eintrei has investigated the effects of two different anesthetic agents on rates of cerebral protein synthesis in rats. In the study of the effects of light thiopental anesthesia, Dr. Eintrei determined rates of protein synthesis in 29 brain regions and found a 12% decrease ($P \leq .05$) in the anesthetized rats in only one brain region, the medial geniculate nucleus. In a similar study of the effects of ketamine anesthesia, Dr. Eintrei determined rates of protein synthesis in 36 brain regions and found significant ($P \leq .05$; 20-26%) decreases in 8 of them. It is noteworthy that the prefrontal and frontal cortex were both significantly affected ($P \leq .01$) under ketamine anesthesia.

Studies of the effects of chronic hypothyroidism in the adult rat on local rates of cerebral protein synthesis have been carried out in collaboration with Dr. D. Dow-Edwards (Dept. Neurosurgery, SUNY Downstate Medical Center). Rates of protein synthesis were determined in 51 brain regions and in the brain as a whole 3 months following surgical thyroidectomy. Significant decreases (25-30%, $P \leq .05$) were found in 13 of the structures, including components of the extrapyramidal motor system, cranial nerve nuclei, and hypothalamic nuclei. The average rate of protein synthesis for the brain as a whole was not significantly affected by thyroidectomy.

In collaboration with Dr. W. J. Schwartz and Dr. T. Scammell (Dept. Neurology, Univ. of Mass.) studies were carried out in order to investigate the effects of circadian rhythm in rats on rates of protein synthesis in the suprachiasmatic nucleus (SCN). The results of these studies show that, in contrast to glucose utilization and electrical activity, rates of protein synthesis are the same in the midpoints of the subjective day and night.

Similarly, no day/night differences in protein synthesis were found in the paraventricular nuclei or in the brain as a whole. These results were presented at the 1988 Annual Meeting of the Society for Research on Biological Rhythms.

The effects of repetitive electrical stimulation of the sciatic nerve on local rates of protein synthesis in the dorsal root ganglia and the spinal cord have been carried out in collaboration with Dr. M. Kadekaro (Dept. Neurosurgery, Univ. of Texas). The results of our initial series of experiments showed that while there was no effect of electrical stimulation on protein synthesis in the dorsal root ganglia, there might be significant effects in the dorsal horn of the spinal cord (+5%, $P \leq .05$) and in Rexed's lamina IX in the ventral horn (-7%, $P \leq .05$). In order to test the validity of this finding, a second series of experiments were carried out this year. The results are currently being analyzed.

Dr. C. Kennedy, in collaboration with Dr. M. Mishkin, Dr. R. Nakamura (Lab. of Neuropsychology), and Dr. C. Gillin (UCSD), has carried out studies on the effects of slow wave sleep on local rates of protein synthesis in the adult rhesus monkey. The results of studies in 3 asleep and 3 awake animals suggest that there are increases in the rates of protein synthesis in a few selective brain regions during periods of slow wave sleep.

The course of normal development in rhesus monkey and in fetal sheep (in collaboration with Dr. R. Abrams, Dept. of Obstetrics, Univ. of Florida) is also being examined. Studies have been carried out in newborn, 25 day, 50 day, and 1 year old monkeys. The results show that over this age range rates of cerebral protein synthesis tend to decrease from birth during the developmental period. Studies have been carried out in fetal sheep beginning with the 118th day of gestation and ending with 5 days after birth. These results are currently being analyzed.

Studies of the effects of testosterone on protein synthesis in regenerating neurons have been carried out with Dr. A. Yu (Dept. of Anatomy, CUNY Medical School). Our initial analysis shows that protein synthesis is increased in regenerating cranial nerve nuclei but that there is no overall effect of testosterone treatment on the nuclei as a whole. We are currently reanalyzing these results and examining rates of protein synthesis at specific levels of the nuclei.

Significance to Biomedical Research and Program of the Institute:

Protein synthesis is probably the most important biochemical process underlying the development, maturation, plasticity, maintenance, and long-term regulation of the nature and degree of functional activity of the nervous system. The structural, functional, and metabolic properties of the tissues largely reflect the role of structural and enzymatic proteins. Peptides that are considered to be neurotransmitters are in some, and possibly all cases, derived from the cleavage of large parent protein molecules. Many hormones within and outside the nervous system are proteins.

It is, therefore, certain that changes in protein synthesis can and do alter function and that some mental and neurological dysfunctions reflect disturbances in this vital biochemical process.

This research is directed at determining the rates of protein synthesis in specific regions of the central nervous system with an ultimate resolution down to the cellular level. This provides for the first time the opportunity to study at the individual structural or anatomical level the changes in protein synthesis that may be the causes, consequences, or correlates of normal conditions, such as maturation, plasticity, differentiation, sleep, learning and memory, behavioral patterns, etc., or pathological conditions, such as hormonal disorders, aging, regeneration in response to injury, convulsive disorders, coma, etc.

Proposed Course:

Studies on the determination of the value of λ and the validation of the [1-¹⁴C]leucine method are being prepared for publication. Manuscripts are being prepared on the results of studies of the effects of hypothyroidism, thiopental and ketamine anesthesia, and circadian rhythms on local rates of protein synthesis. Results of studies of the effects of electrical stimulation, development, sleep, and testosterone in regenerating nuclei continue to be analyzed.

Further experiments will be carried out to investigate the stability of the value of λ with respect to different brain regions and various plasma levels of leucine.

Publications:

Holcomb HH, Links J, Smith C, Wong D. Positron emission tomography: measuring the metabolic and neurochemical characteristics of the living human nervous system. In: Andreasen NC, ed. Brain Imaging: implications for Psychiatry. Washington, DC: Amer. Psychiatric Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00903-11 LCM
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Purification and Identification of Brain Proteinases and their Cleavage Products		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	G. E. Deibler	Research Chemist LCM, NIMH
Others:	M. W. Kies	Chemist LCM, NIMH
COOPERATING UNITS (if any) Multiple Sclerosis Research Center, Georgetown Univ. Med. Center, Washington, D.C. (J.R. Richert); Lab. of Cereb. Metab., State Univ. of NY Health Sci. Center of Brooklyn, NY (D. Dow-Edwards)		
LAB/BRANCH Laboratory of Cerebral Metabolism		
SECTION Section on Developmental Neurochemistry		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>After reviewing the literature on brain proteinases, we have decided to isolate <u>calcium activated neutral proteinases (CANPs)</u>, to identify their bond specificity and to characterize their cleavage products. The objective and proposed methods are described on the next page.</p> <p>Since we have a valuable collection of <u>myelin basic proteins (MBPs)</u> and <u>peptides</u> derived from them, our collaboration with Dr. John Richert has resulted in the identification of <u>ten different T-cell reactive sites</u>. These identifications were made by studying the proliferative response of forty MBP-reactive T-cell clones which were isolated from a multiple sclerosis patient.</p> <p>Our collaboration with Dr. Diana Dow-Edwards on the effect of <u>aspartame</u> on the developing guinea pig fetus has resulted in a possible correlation with the increase of plasma phenylalanine and <u>functional brain development</u>.</p>		

Project Description:Objectives:

1. The isolation and stabilization of calcium-activated neutral proteinase (CANP) from brain and a study of its bond specificity with known CNS proteins and/or peptides as substrates.
2. Completion of the analyses of the guinea pig plasma for aspartic acid and phenylalanine and correlation of the increase of phenylalanine with diminished brain function.
3. Honing in on the T-cell active sites by using synthetic peptides and then using these active peptides to test MBP-reactive T-cells from normal and other multiple sclerosis patients.

Methods Employed:

1. CANP will be precipitated with ammonium sulfate from the supernatant fraction of total brain homogenate or detergent-solubilized myelin. Optimal stabilization conditions will be investigated - e.g., addition of phospholipid (reported to stabilize CANP from red blood cells); use of proteinase inhibitors, reducing agents and EDTA or EGTA. The dissolved ammonium sulfate precipitate will be subjected to ion-exchange chromatography on FPLC. In the final step of purification, a phenyl-Sepharose column (which separates proteins according to their hydrophobicity) will be used. Purity will be assessed by SDS polyacrylamide electrophoresis and HPLC. Activity will be determined by the standard casein assay. Purified preparations of known CANP activity will be used to cleave proteins of known primary structure. Their cleavage products will be located and purified on HPLC. Amino acid and end-terminal analyses will be used to identify the cleavage products and establish the bond specificity of CANP.
2. A sensitive amino acid analysis using 20 μ l samples has been developed and shortened to quantitate the plasma samples for the protein synthesis work and the aspartame collaboration.
3. A limited thrombic digestion of synthetic peptide 86-105 will be used to cleave this peptide approximately in half. HPLC will be used to purify the digestion products and amino acid analysis to determine their compositions.

Major Findings:

Despite wide acclaim for use as an artificial sweetener, aspartame is potentially harmful to the developing fetuses in guinea pigs and humans because brain development occurs in utero. Phenylalanine has been shown to cause most of the toxic effects of aspartame. When aspartame (500 mg/kg/day) is administered to pregnant guinea pigs, phenylalanine levels in the plasma

of the moms and their pups is increased approximately 10%. The guinea pig pups show loss of cognitive response which is indicative of a poorly developed brain.

MBP-specific human T-cell clones can be isolated from patients with multiple sclerosis and normal humans. By studying the proliferative response of the reactive T cells from a multiple sclerosis patient, we have found forty MBP-specific human T cell clones. Thirty clones responded to human myelin basic proteins (HBP) fragment 98-170, seven recognized fragment 1-97 and three did not proliferate in response to either fragment. In studies with xenogeneic MBPs isolated from rabbit, guinea pig, rat, cow and chicken, four different patterns of reactivity were seen with the clones which recognized 98-170, three with those that responded to 1-97 and three which recognized neither fragment. The most common pattern of reactivity, expressed by eighteen of the clones, consisted of recognition of HBP fragment 98-170 and all xenogeneic MBPs tested with the exception of chicken MBP. There are possibly ten MBP-reactive sites on the human MBP.

Significance to Biomedical Research:

This basic research on the effect of moderate and toxic doses of aspartame in pregnant guinea pigs will evaluate the threat to human fetuses of increasingly greater quantities of aspartame consumed by pregnant women.

While the direct correlation between BP-sensitivity and demyelination in multiple sclerosis (MS) patients has never been established, an autoimmune reaction to this antigen remains the most probable explanation for the development of MS. The current studies have shown that the T-cells of one MS patient demonstrate a very complex pattern of reactivity to MBP peptides. Similar studies in normals and other patients will clarify whether this T-cell reactivity is unique and related to disease activity.

Publications:

Richert JR, Reuben-Burnside CA, Deibler GE, Kies MW. Peptide specifications of myelin basic protein human T cell clones, Neurology 1988;38:739-742.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02216-05 LCM
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Metabolic Mapping of the Brain during Rewarding Self-Stimulation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	L. J. Porrino	Guest Researcher LCM, NIMH
Others:	L. Sokoloff	Chief LCM, NIMH
COOPERATING UNITS (if any) Department of Pharmacology and Psychiatry, Boston University School of Medicine, Boston, MA (C. Kornetsky).		
LAB/BRANCH Laboratory of Cerebral Metabolism		
SECTION Section on Developmental Neurochemistry		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.00	PROFESSIONAL: 1.5	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) The <u>deoxyglucose</u> method is being used to study alterations in local cerebral glucose utilization resulting from <u>rewarding electrical brain self-stimulation</u> to discrete brain sites as well as resulting from the administration of drugs of abuse. By mapping metabolic activity in rats under these conditions, information can be obtained about those areas of the brain involved in <u>motivation</u> and <u>reinforcement</u> .		

Project Description:Objectives:

The goal of the present project is to map those regions of the rat brain activated during positive reinforcement processes. Both rewarding electrical brain stimulation and the administration of psychostimulants known to produce rewarding effects are being studied.

Methods Employed:

The 2-[¹⁴C]deoxyglucose method permits mapping of functional neural pathways simultaneously in all anatomical components of the central nervous system, allowing the identification of complex neural circuits functionally active during pharmacological and behavioral manipulations.

The standard protocols for the quantitative autoradiographic 2-[¹⁴C]deoxyglucose method were used in freely moving rats following administration of pharmacological agents and/or during electrical intracranial self-stimulation. Behavioral assessments included: 1) measurement of locomotor activity, 2) observational rating scales, and 3) measurement of rates of self-stimulation.

Major Findings:

- A. Experiments comparing the effects of different rates of administration of the abused drug, cocaine, have been conducted. Four groups of animals were studied: 1) intravenous cocaine (1.0 mg/kg); 2) intrapentoneal cocaine (10 mg/kg); 3) saline (intravenous), and 4) saline (intrapentoneal). No differences in glucose utilization were observed in saline injected animals. Cocaine administration resulted in increases in metabolic activity in the substantia nigra reticulata and globus pallidus regardless of route of administration. Alterations in functional activity in the nucleus accumbens and prefrontal cortex occurred only following intravenous cocaine administration. Locomotor activity was similar in cocaine-treated groups.
- B. Experiments to identify the sites of action of the reward-enhancing effects of cocaine as measured in self-stimulation paradigms were conducted in collaboration with Dr. Conan Kornetsky of the Behavioral Pharmacology Laboratory, Boston University School of Medicine. Significant interactions between the effects of cocaine and rewarding brain stimulation in local cerebral metabolic activity were found in the nucleus accumbens and the olfactory tubercle.

Significance to Biomedical Research and the Program of the Institute:

These experiments have used metabolic mapping methods to examine different aspects of the behavioral and pharmacological actions of cocaine, one of the most widely abused drugs in the U.S. at present. These data provide converging evidence for the importance of the mesocorticolimbic system as the neural substrates of the reinforcing properties of cocaine.

Proposed Course:

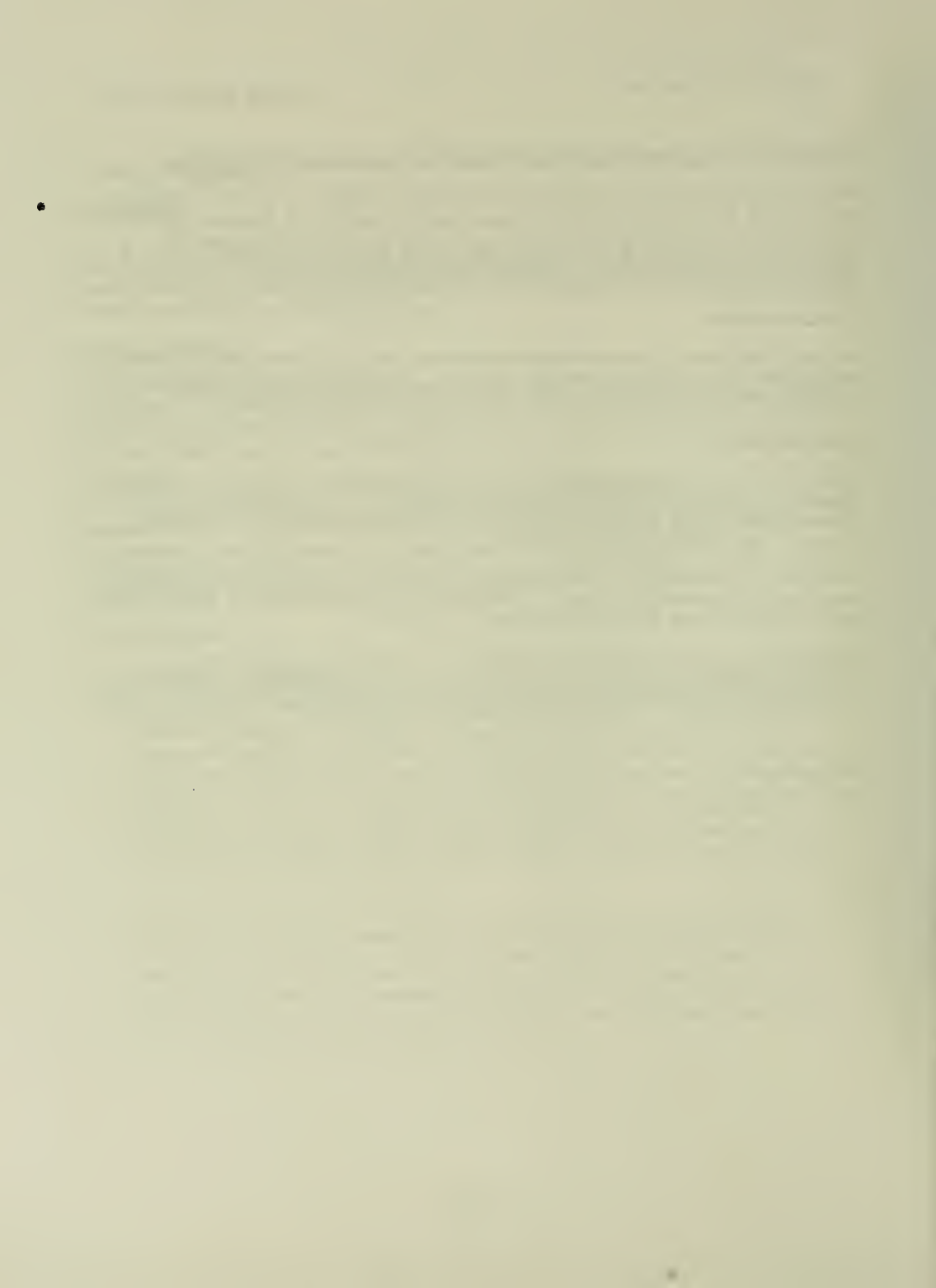
We will continue to test the hypothesis that drugs of abuse have a common mechanism of action in producing euphoria. Drugs from other pharmacological classes such as the opiates will be tested in these paradigms.

Publications:

Porrino LJ. Using the quantitative 2-[¹⁴C]deoxyglucose method for metabolic mapping of the brain during reinforced behavior. In: Dahlstrom A, ed. Proceedings of the VI International Catecholamine Symposium. Jerusalem, Israel, June 14-19, 1987), in press.

Porrino LJ, Kornetsky C. The effects of cocaine on local cerebral metabolic activity. In: Brown RA, Clonier D, Ashgar K, eds. Mechanisms of cocaine abuse and toxicity. NIDA Research Monograph, in press.

Porrino LJ, Damer FR, Crane AM, Sokoloff, L. Selective alterations in cerebral metabolism within the mesocorticolimbic dopaminergic system produced by acute cocaine administration in rats, Neuropsychopharmacol 1988;1:109-18.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02217-05 LCM												
PERIOD COVERED October 1, 1987 to September 30, 1988														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Plasticity in the Developing Monkey Visual System														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: C. B. Smith</td> <td style="width: 33%;">Research Chemist</td> <td style="width: 33%;">LCM, NIMH</td> </tr> <tr> <td>Others: L. Sokoloff</td> <td>Chief</td> <td>LCM, NIMH</td> </tr> <tr> <td>K. D. Pettigrew</td> <td>Res. Math. Statistician</td> <td>TSMB, NIMH</td> </tr> <tr> <td>S. Herdman</td> <td>Guest Researcher</td> <td>LCM, NIMH</td> </tr> </table>			PI: C. B. Smith	Research Chemist	LCM, NIMH	Others: L. Sokoloff	Chief	LCM, NIMH	K. D. Pettigrew	Res. Math. Statistician	TSMB, NIMH	S. Herdman	Guest Researcher	LCM, NIMH
PI: C. B. Smith	Research Chemist	LCM, NIMH												
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K. D. Pettigrew	Res. Math. Statistician	TSMB, NIMH												
S. Herdman	Guest Researcher	LCM, NIMH												
COOPERATING UNITS (if any) Department of Neurology, Johns Hopkins Medical School, Baltimore, MD (R. Tusa)														
LAB/BRANCH Laboratory of Cerebral Metabolism														
SECTION Section on Developmental Neurochemistry														
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892														
TOTAL MAN-YEARS: 0.8	PROFESSIONAL: 0.5	OTHER: 0.3												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>The postnatal development of the central visual pathways depends on the quality of the visual environment. During the critical period in the primate visual system environmental manipulation can modify the physiological properties of visual cortical cells. The purpose of this project is to study the underlying biochemical events that imbue the nervous system with the property of <u>plasticity</u>. <u>Protein synthesis</u> is a biochemical process which is involved in bringing about changes in morphology, adjustments in growth rates, and remodeling and maintenance of structures. We have, therefore, used the <u>[¹⁴C]leucine method</u> to study the relationships between local plastic changes which occur in the <u>developing monkey visual system</u> and local rates of protein synthesis.</p>														

Project Description:Objectives:

The purpose of this project is to study the biochemical events associated with plasticity. The developing rhesus monkey visual system has been chosen as a model system because the physiological and anatomical responses to deprivation have been so well described by others. We have focussed initially on the process of protein synthesis because it is a requirement for growth and development and because changes in morphology and rates of growth and remodeling and even maintenance of existing structures should be reflected in changes in rates of protein synthesis.

The postnatal development of the central visual pathways depends on the quality of the visual environment. During the critical period alterations in the conditions of visual input can modify the physiological properties of visual cortical cells. If a monkey is monocularly deprived during the first few weeks of life there is a reorganization of the striate cortex such that the ocular dominance columns representing the functioning eye extend beyond their boundaries and broaden at the expense of the adjacent columns representing the deprived eye. Eventually, most of the striate cortex may be incorporated into a monocular visual system that serves only the deprived eye. The organization of the lateral geniculates (dLGN), the locus of the cell bodies of the terminals in striate cortex, remains normal.

Specific aims for this fiscal year:

1. To complete the studies of the effects of acute monocular occlusion on local rates of protein synthesis in the laminae of the dLGN in 50 day old rhesus monkeys.
2. To continue studies in collaboration with Dr. R. Tusa (Department of Neurology, Johns Hopkins) on the effects of reverse-suture on eye movements.
- 3) To determine the effects of chronic monocular deprivation at the end of the critical period (from 36 to 50 days) on local rates of protein synthesis in the laminae of the dLGN.

Methods Employed:

Local rates of cerebral protein synthesis were determined with the [$1\text{-}^{14}\text{C}$]leucine method (Smith et al., J Neurosci 1984;4:2489-2496).

Unilateral or bilateral tarsorrhaphies were performed under ketamine anesthesia on newborn and 25 day old rhesus monkeys. In some animals a reverse-suture paradigm was used; i.e., unilateral lid suture was performed at birth, and at 25 days the sutured eye was opened and the other eye was closed.

Eye movements were recorded with scleral search coils that were implanted at the time of the reverse-suture.

Major Findings:

The acute monocular occlusion studies in 50 day old monkeys have been completed and Dr. Pettigrew has carried out the statistical analyses of all of the results of these studies. Rates of protein synthesis determined in the six laminae of left and right LGN in acutely and chronically monocularly occluded, age-matched monkeys were subjected to an analysis of variance with repeated measures on 3 factors: group, laminae, and condition. Results of these analyses for both 25 and 50 day old monkeys were similar. There were no significant 3 way interactions. At both ages there was a significant interaction of group (acute v. chronic) and conditions (occluded v. nonoccluded). The profiles, however, appeared to be qualitatively different for the two age groups. At 25 days of age the nondeprived (chronic) laminae had values of protein synthesis that were similar to the nonoccluded (acute) laminae. The deprived (chronic) laminae, however, had lower rates of protein synthesis than the occluded (acute) laminae. At 25 days of age, the effect of chronic deprivation, therefore, appears to be on the deprived laminae. At 50 days of age the profiles were more complicated. The nondeprived (chronic) laminae had higher rates of protein synthesis than the nonoccluded (acute) laminae and the deprived (chronic) laminae had lower rates of protein synthesis than the occluded (acute) laminae. The effect of chronic deprivation at this age appears to be on both deprived and nondeprived cells. In order to test whether or not this apparent difference in the profiles at these two ages is statistically significant Dr. Pettigrew carried out an analysis of variance on all of the data with repeated measures on 4 factors: group (acute v. chronic), condition (occluded v. nonoccluded), age (25 day v. 50 day), and laminae. While there was a highly significant interaction between group and condition, there was no significant interaction of age, group and condition. This analysis shows that the apparent difference in the profiles is not statistically significant and that at both ages, 25 and 50 days, the response to chronic monocular deprivation is similar.

Studies of the nystagmus that develops with reverse-suture have continued with Dr. R. Tusa (Johns Hopkins). Dr. Tusa has found that visual acuity is severely affected in both eyes following this procedure.

Significance to Biomedical Research and the Program of the Institute:

Plasticity, the capacity of the nervous system to respond to changes in the environment, is one of the most fundamental properties of nervous tissue. Learning, a form of plasticity, is a process of intense interest to neurochemists the world over. In an attempt to study some of the biochemical processes underlying plastic changes, we have embarked on this study of the developing monkey visual system about which the physiology and anatomy are well known. Studies with the [^{14}C]leucine method for local rates of protein synthesis and the [^{14}C]deoxyglucose method for local rates of glucose utilization are directed at first a description of some of the biochemical events which occur and then a determination of the regulation of these

events. The understanding of these events may provide some insight into the unique properties of the critical period which make it so responsive to environmental manipulation. In addition, this research may have some direct implications on the clinical management of children with congenital cataracts and strabismic amblyopia.

Proposed Course:

Results of experiments on deprivation from 36 to 50 days will be analyzed. Manuscripts are being prepared on the results of monocular and binocular deprivation studies.

Publications:

Herdman SJ, Tusa RJ, Smith CB. Cortical areas involved in OK and VOR in cats: I. Metabolic activity. J Neurosci 1988 (in press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02220-05 LCM

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regional Biochemical Changes in the Normal Aging Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. B. Smith

Others: L. Sokoloff

Chief

LCM, NIMH

E. Palombo

Visiting Fellow

LCM, NIMH

Y. Sun

Guest Researcher

LCM, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Developmental Neurochemistry

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Studies have been carried out on the effects of aging on cerebral protein synthesis and glucose utilization in rats. With the application of local methods developed in this Laboratory, discrete regions of the brain can be examined in normal conscious animals. The regional changes in glucose utilization indicate that entire sensory pathways are affected by the aging process. The fact that similar changes are found in the same pathways with respect to protein synthesis suggests that some of these changes reflect an adaptation of the nervous system to a chronic lack of input. The age-dependent decreases in glucose utilization found in the striatum have been followed up with studies of the effects of aging on the metabolic responsiveness to the dopaminergic agonist, apomorphine.

Project Description:Objectives:

The overall purpose of these studies is to examine the effects of normal aging in rats on regional rates of metabolic processes in the brain. Our previous studies have shown that decreases in rates of both glucose utilization and protein synthesis occur with age in the brain as a whole and in selective brain regions. Changes in both processes were found in components of the primary visual and auditory systems. These effects might be the consequences of a chronic lack of sensory input due to age related degenerative changes in both retina and inner ear. One of the most statistically significant decreases in glucose utilization was found in the striatum. The major objective of the ongoing work during this fiscal year was to study the functional consequences of senescent changes in the nigrostriatal dopaminergic system by determining the effects of normal aging on the metabolic responsiveness of dopamine-receptor activation by systemically-administered apomorphine.

Methods Employed:

In this study Fisher 344 male rats were obtained from the colony maintained by the National Institute on Aging. Three age groups were studied: young adults, 4-6 months of age; middle-aged rats, 14-16 months of age; and old rats, 23-25 months of age. The [^{14}C]deoxyglucose method (Sokoloff et al., 1977) was used to determine local rates of cerebral glucose utilization. Rats were administered with apomorphine at 0.5 mg/kg, 1.5 mg/kg or 5.0 mg/kg or normal saline vehicle 10 minutes before the administration of [^{14}C]deoxyglucose. Behavioral and physiological responses were monitored throughout the study.

Major Findings:

The results of our pilot study showed significant dose-dependent effects of apomorphine in 6 of the 14 brain regions examined in the young adult rats. Age-dependent changes in responsiveness were found in 3 of the 6 regions. Studies have been completed in 72 rats. The integrated plasma and tissue specific activities for deoxyglucose has been calculated for each animal. The autoradiographs of brain sections have been prepared and 108 of the resulting 216 films have been analyzed on our image processing system. Rates of glucose utilization are being determined in 47 brain structures and in the brain as a whole.

Proposed Course:

Completed experiments on the effects of aging on the metabolic responsiveness to apomorphine continue to be analyzed. Dr. Sun, who has just joined our group as a Guest Researcher, is being trained to carry out the densitometry.

The addition of her efforts and a second image-processing system which is currently being installed in the laboratory should accelerate this labor-intensive aspect of the work. When the analysis is completed a manuscript of this work will be prepared.

Publications:

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02307-03 LCM
PERIOD COVERED October 1, 1987 to September 31, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Role of Proteinases in Production and Control of Neuropeptides		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: M. W. Kies	Chemist	LCM, NIMH
Others: G. E. Deibler	Research Chemist	LCM, NIMH
COOPERATING UNITS (if any) Department of Pathology, University of Washington, Seattle (E. C. Alvord, Jr.)		
LAB/BRANCH Laboratory of Cerebral Metabolism		
SECTION Section on Developmental Neurochemistry		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Since returning to work after a long illness, I have devoted much of my time to helping with the preparation of the manuscripts listed below. Their significance to biomedical research will be discussed later in the report.</p> <p>1. LIPOPOLYSACCHARIDE (LPS) AUGMENTS ADOPTIVE TRANSFER OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN THE LEWIS RAT, by T. Hamada, B.F. Driscoll, M.W. Kies and E.C. Alvord, Jr. (Submitted for publication.)</p> <p>2. ROLE OF PHOSPHORYLATION IN CONFORMATIONAL ADAPTABILITY OF BOVINE MYELIN BASIC PROTEIN, by G.E. Deibler. A.L. Stone and M.W. Kies. (Submitted.)</p> <p>3. EVIDENCE FOR MULTIPLE HUMAN T CELL RECOGNITION SITES ON MYELIN BASIC PROTEIN, by J.R. Richert, C.A. Reuben-Burnside, G.E. Deibler, R.E. Martenson, L.J. Dragovic and M.W.Kies. (Submitted.)</p> <p>4. EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN NON-HUMAN PRIMATES: AN EXCELLENT MODEL FOR MULTIPLE SCLEROSIS, by E.C. Alvord, Jr., L.M. Rose, S. Hruby, T.L. Richards, R. Petersen, C.-M. Shaw, E.A. Clark, L.H. Ericsson, W.A. Steward, D.W. Paty and M.W. Kies. (This paper will appear as a chapter in a book on "Biomedical Research on Primates.")</p>		

Project Description:Objective:

The broad objective of this project is to study mechanisms of proteolysis in the nervous system, to improve our understanding of cellular metabolism and control of neurotransmission. After an extensive review of the literature on proteinases and their role in the CNS, the decision was made to concentrate on calcium-activated neutral proteinase (CANP). Its occurrence in the nervous system has been well-established and details for its isolation and assay are available in the literature. Several different functions have been ascribed to CANP, based on in vitro studies, but it remains to be seen how important these activities are in vivo. Its potential for effecting the post-translational modification of prohormones and polypeptides, suggests a possible role for CANP in the production of neuropeptides.

Methods:

Details of methodology will be given in project #Z01 MH 00903-11 LCM. As soon as we are able to recruit technical help, isolation of CANP will be undertaken.

Major Findings:

This report is a continuation of my 1985-86 report, with an intervening extended absence. Because of this absence due to illness, I have been unable to devote any effort to the study, except for the collection of an extensive bibliography with concurrent planning of experimental approaches which might be feasible.

Significance to Biomedical Research and the Program of the Institute:

Characterization of specific proteinases and demonstration of their possible functions in vivo is of general significance to biomedical research. The proposed studies will contribute to the program of the Institute by enhancing our understanding of the production and control of the neuropeptides involved in nerve transmission.

The manuscripts recently prepared for publication represent major contributions to research in neuroscience and immunology: 1. In the first study, we showed that LPS enhances adoptive transfer of EAE by stimulation of non-specifically sensitized cells; our results provide a unique strategy for potentiation of other (beneficial) types of adoptively transferred T cell-mediated immunity, such as protection against viral infection and anti-tumor immunity. 2. In the second study, we developed a novel procedure for the isolation of a homogeneous monophosphorylated form of myelin basic protein. For the first time it was possible to show unambiguously that the presence of phosphate in the BP molecule increased its stable beta-structure. 3. The third study addressed the identity of the human encephalitogenic site in myelin basic protein, which has never been determined. Cloned BP-specific

cells isolated from an MS patient were assayed for their proliferative response to purified BPs and related peptide fragments. We were able to show that the number of unique sites in the BP molecule is at least 10. While these results per se do not determine the encephalitogenic site, they will eventually enable us to identify the site in myelin basic protein responsible for autoimmune damage in the CNS resulting from human idiopathic demyelinating disease. Our preparations of highly purified, well-characterized proteins and peptides were absolutely essential for the study. 4. This paper is a review of our collaborative work on the induction and prevention of EAE in non-human primates. It encompasses clinical, pathological, biochemical, immunological, radiological and therapeutic studies. The breadth and scope of the data resulting from the collaboration between our two laboratories provides a unique data base for developing treatment protocols for human idiopathic demyelinating diseases.

Proposed Course:

Isolation and characterization of CANP from myelin or, if that is not feasible, from whole white matter. Our first goal will be to develop a technique for stabilizing the isolated enzyme. Secondly, we will examine its peptide bond-splitting specificity with proteins of known amino acid sequence.

Publications:

Alvord EC Jr, Compston DAS, Kies MW. Is multiple sclerosis already being prevented? In: Confavreux C, Aimard G, Devic M, eds. Trends in European multiple sclerosis research. Amsterdam: Elsevier Science Publishers b.v., 1988;61-65.

Alvord EC Jr, Rose LM, Hruby S, Richards TL, Peterson R, Shaw CM, Clark EA, Ericsson LH, Stewart WA, Paty DW, Kies MW. Experimental allergic encephalomyelitis in non-human primates: an excellent model of multiple sclerosis. In: Jonker M, ed. Biomedical research in primates. Amsterdam: Elsevier Science Publ, 1988 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02308-03 LCM
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Growth and Development of Dopaminergic Neurons		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> PI: B. F. Driscoll Research Biologist LCM, NIMH </div>		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Cerebral Metabolism		
SECTION Section on Developmental Neurochemistry		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: <div style="text-align: center;">1.5</div>	PROFESSIONAL: <div style="text-align: center;">1.0</div>	OTHER: <div style="text-align: center;">0.5</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) <p> <u>Survival and development of dopaminergic neurons</u> from <u>embryonic rat mesencephalon</u> were examined in <u>dissociated cell cultures</u>. Survival and the degree of development were assessed by measuring the amount of labelled dopamine taken up by the <u>high affinity dopamine reuptake system</u>. The development of this system correlates well with the development of the dopaminergic neurons. </p> <p> <u>Soluble factors</u> in the culture medium play a dominant role in the survival, development and subsequent maintenance of these cells <u>in vitro</u>. Removal of these factors from young cultures (one week <u>in vitro</u>) appears to disrupt all the cells in culture. Removal from more mature cultures (two weeks or more <u>in vitro</u>) did not cause obvious morphologic damage but did cause the rapid inactivation or loss of the dopamine reuptake system. The more developed dopaminergic neurons apparently depend on soluble factors for continued survival or to maintain a functioning reuptake system. </p>		

Project Description:Objective:

To determine the factors involved in development of central nervous system pathways by observing survival and development of neurons in dissociated cell cultures.

Methods:

Brain tissue is removed from embryos and separated into specific regions. Single cell suspensions are prepared from each region and cultured in vitro. There is only a narrow period of time when post-mitotic neurons can be prepared for in vitro culture. Since each brain region passes through this period at its own unique time during development, embryos of various ages are required.

The cells were cultured in serum-free medium. The two types of serum-free medium used were both made in a mixture of Dulbecco's MEM and Ham's F-12 medium. The first type (enriched) contains added insulin, transferrin, selenium, progesterone, putrescine and a relatively large amount of bovine serum albumin complexed with linoleic acid. This last addition is the single largest source of protein in the medium and potentially the major source of contaminating factors. Therefore, in most recent experiments we have used a second, more simplified type of serum-free medium. It consists of medium with added insulin, transferrin and selenium. Direct comparison indicates that higher levels of unknown growth factors are present in the enriched medium.

We are interested in assessing the development of mesencephalic dopaminergic neurons and the effect that various cells or factors have on this development. The degree of development was determined by quantitating the level of dopamine uptake by the reuptake system which is present in all dopaminergic neurons. The uptake of ^3H -labelled dopamine was measured by liquid scintillation counting or (for qualitative morphologic studies) by autoradiography. To identify and quantitate the cell types present, representative cultures were fixed, permeabilized and exposed to antibodies which recognize various cell-type specific antigens, particularly antigens present in neurons or astroglia. After exposure to a second antibody containing a fluorochrome, the cultures were examined by fluorescence microscopy.

Major Findings:

In order to detect and analyze any change in the survival or development of the dopaminergic neurons in vitro, an in-depth analysis of the kinetics of the dopamine reuptake system has been performed. Also, the role of soluble factors has been assessed using culture conditions which limit the inadvertent addition of exogenous factors.

Uptake of free dopamine by the cells in culture is not via a single reuptake system. Besides the high affinity dopamine reuptake system characteristic of catecholnergic neurons, several lower affinity systems also appear to be operating. However, by selecting the appropriate concentration of dopamine and assaying for a short period of time, the bulk of the reuptake is via the high affinity system. This system is found only in cultures derived from the mesencephalon. Changes in the amount of dopamine taken up under these conditions are an accurate indication of the survival and development of dopaminergic neurons in vitro. In contrast, use of higher concentrations of dopamine along with longer incubation times leads to uptake that may not be neuronal but some of which could be specific to particular brain regions.

Observation of the effects of added or endogenously generated factors on the development of the dopaminergic neurons has been aided by the use of a minimal growth medium. Under these conditions any necessary growth/survival factors must be added to the culture or endogenously generated. A clear role for soluble factors in the development and maintenance of the dopaminergic neurons can be demonstrated. Removal of soluble factors from young cultures (one week or less in vitro) results in visible morphologic changes in the cultures accompanied by a large decrease in the activity of the dopamine reuptake system. Removal of factors from older cultures (two weeks or longer in vitro) results in little or no visible morphologic effect on the culture but the activity of the dopamine reuptake system drops dramatically. Whether this represents damage to the dopaminergic neurons or impairment of the reuptake system (with sparing of the neurons) is currently not known.

As the mesencephalic neurons develop in vivo, their response to soluble factors changes. Complete removal of soluble factors from embryonic day 14 mesencephalic cells does not affect development of these cells unless it is done at frequent intervals. In contrast, complete removal of soluble factors from embryonic day 15 mesencephalic neurons at any time during culture results in the rapid inactivation or loss of the dopamine reuptake system.

Proposed Course:

Further studies are required to determine the nature and regional specificity of the lower affinity dopamine reuptake systems. In particular, we would like to know the identity of the cell type responsible for reuptake, the dopamine concentration at which the reuptake occurs, and whether these reuptake systems are found throughout the brain or are restricted to specific regions.

Further studies are also required to determine the nature and mode of operation of soluble factors responsible for the development and maintenance of the dopamine reuptake system or the dopaminergic neurons. So far, the data indicate that soluble factors are required for development (early in culture) and maintenance (late in culture). Whether these represent activities of separate factors or the same factor is unknown. This type of experiment can address the question of whether the neurons die when deprived of necessary factors or simply become inactive. If the latter is correct, there might be a period of time after these cells malfunction but before

they die when they could be reactivated by exposure to soluble factors.

As part of an ongoing collaboration within the laboratory, we will continue to provide various types of neuronal and glial cultures for studies on CNS energy metabolism. These cultures will also be available for use in other studies where in vitro manipulation is required.

Significance to Biomedical Research and the Program of the Institute:

The central nervous system dopaminergic neurons, although few in number, project widely and influence a large number of CNS activities. In particular, deficits in the dopaminergic neurons have been implicated as playing a role in some of the most devastating neurological disorders of humans. In many of these cases, the dopaminergic neurons may function to maintain a tonic state in a particular region and a simple increase in the number of dopamine producing neurons might be beneficial. However, treatments of this type require a complete understanding of how dopaminergic neurons develop and function. Of particular importance is an understanding of the factors responsible for the survival and differentiation of dopaminergic neurons and an understanding of mechanisms responsible for controlling the level of neurotransmitters maintained in the surrounding tissue. The results of studies reported here should prove helpful in answering these questions.

Recent studies indicate that during the development of neurologic disorders there may be a period of time between the onset of neuronal malfunction and the actual death of the cell. Clinical intervention during this interval could be therapeutic. Restoration of some factor(s) required for cell function and survival could prevent cell death, restore function and reverse clinical signs. Results of studies on the role and identification of soluble factors that are active in vitro could be of direct use in the clinical setting.

Publications:

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02414-01 LCM
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Metabolic Interdependence of Neurons and Glia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	E. C. Kaufman	Research Chemist LCM, NIMH
Others:	B. F. Driscoll	Research Biologist LCM, NIMH
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Cerebral Metabolism		
SECTION Section on Developmental Neurochemistry		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.6	PROFESSIONAL: 1.0	OTHER: 0.6
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) In this project we are investigating the <u>interrelationship</u> of <u>neurons</u> and <u>glia</u> with special emphasis on enzymatic reactions unique to glial cells and on the interdependence of essential metabolic pathways between these two types of cells. Primary cultures of neurons and glia derived from various areas of the brain at different stages of development will be used in these studies.		

Project Description:Objectives:

The initial objective of this project is the localization and characterization of CO₂ fixation in glia, neurons and fibroblasts. Determination of 1) factors which regulate this important anapleoric reaction and 2) identification of the ultimate products will be undertaken. We will also attempt to determine whether the products formed in glial cells as a result of this reaction are exported from the glia and serve as substrates for essential processes in neighboring neuronal cells.

Methods:

Primary cultures of neuronal and glial cells have been prepared and grown according to standard procedures. Labeling with appropriate antibodies has been used to determine the purity of the cultures. Both fetal and newborn brains have been used as a source of these cells. CO₂ fixation has been measured by determining the acid stable [¹⁴C]labeled products formed after incubation of cells with [¹⁴C]NaHCO₃. Thin-layer and paper chromatography have been used to separate these products. Since pyruvate carboxylase, the enzyme responsible for the major part of CO₂ fixation in brain, is a mitochondrial enzyme parallel studies on isolated brain mitochondria have been carried out.

We have shown that increasing the potassium concentration in the medium from 5 to 55 mM will produce a significant increase in CO₂ fixation in glial cells. A similar increase in CO₂ fixation is observed when isolated rat brain mitochondria are incubated with 55 mM potassium rather than 5 mM potassium.

Preliminary examination of the effect of increased potassium concentration on the distribution of labeled products (following exposure to ¹⁴CO₂) between the glial cells and the surrounding medium does not indicate any effect of potassium on the fraction of the newly formed ¹⁴C-labeled products released from the glial cells.

We have confirmed an earlier observation that CO₂ fixation takes place primarily in glial cells. Some CO₂ fixation can be observed in fibroblasts, but the CO₂ fixation in fibroblasts is not responsive to increased potassium.

Significance to Biomedical Research and the Program of the Institute:

Pyruvate carboxylase in liver and kidney has been studied extensively and its role as the enzyme catalyzing the first step in gluconeogenesis is well established. The role of this enzyme in brain is less well understood. For example, in brain its role may be mainly anapleoric rather than gluconeogenic. Patients with an inborn error of metabolism in which pyruvate carboxylase actively is either very low or missing have severe disorders of the central nervous system. There is a depletion of cerebral cortical neurons, gliosis and other degenerative changes such as marked reduction in

cerebral white matter. Mental retardation, generalized seizures, and dystonic movements have also been observed. All of these observations suggest that this enzyme plays a critical role in the function of the cerebral nervous system and that when this enzyme is missing severe problems develop.

Factors which control pyruvate carboxylase in brain have not been well characterized. It is known that potassium stimulates purified pyruvate carboxylase isolated from brain. We have examined the effect of varying the sodium and potassium concentrations in the medium in which glial cells are incubated. These experiments demonstrated that increasing the potassium concentration and lowering the sodium concentration will increase the rate of CO_2 fixation in glial cells.

There is evidence the potassium released into the intercellular space following nerve stimulation is taken up by the neighboring glial cells. This uptake of potassium by astrocytes is believed to serve several purposes. One is simply to protect neurons against large changes in the concentration of extracellular potassium. Secondly, it has been proposed that glial cells may respond metabolically to changes in extracellular potassium and that the release of potassium by nerve cells may constitute a signal to the surrounding glial cells. Pentreath and Kai-Kai (Nature, 1982) have demonstrated an effect of both nerve stimulation and increased potassium on glycogen formation. Similarly, our results indicate that the anapleoric reaction catalyzed by pyruvate carboxylase may also respond to the increased potassium which results from nerve stimulation. Both of these reactions may be important in maintaining a constant energy supply and in replenishing the 3 and 4 carbon units necessary for the synthesis of the amino acid transmitters, GABA and glutamate.

Proposed Course:

We will continue to investigate the role of pyruvate carboxylase and of CO_2 fixation in general in the central nervous system. There will be special focus on the localization of this reaction. Since this reaction is known to have two roles; i.e., gluconeogenic or anapleoric, both of these functions will be studied. The possibility that this reaction contributes to glycogen synthesis in glial cells will also be investigated.

Since pyruvate transport is a necessary first step in pyruvate metabolism factors affecting this process will also be investigated.

Publications:

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02431-01 LCM
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Intracellular Mechanisms of Carbohydrate Transport and Metabolism in Neurons & Glia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	T. Nelson	Medical Officer (Research) LCM, NIMH
Others:	L. Sokoloff	Chief LCM, NIMH
	N. Eng	Chemist
	J. Kline	Biol. Lab. Tech. LCM, NIMH
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Cerebral Metabolism		
SECTION Section on Developmental Neurochemistry		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.00	0.5	0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>This report describes initial studies which have been undertaken in a new project which will examine the <u>cellular and intracellular mechanisms</u> of <u>carbohydrate transport</u> and <u>metabolism in neurons and glia</u>. Inasmuch as this is a new area of investigation for this laboratory, a number of preliminary experiments were necessary to establish and to determine optimal conditions for some of the assays to be used in the experiments.</p> <p>Brain and liver microsomes have been prepared and used to determine glucose-6-phosphatase(G-6-Pase) activity on glucose-6-phosphate(G-6-P) and deoxyglucose-6-phosphate(DG-6-P) under a variety of conditions. The microsomes will be used ultimately to determine the mechanisms of the transport of <u>glucose</u> and/or <u>deoxyglucose</u> from the cisterns of the <u>endoplasmic reticulum</u> following hydrolysis of G-6-P and DG-6-P.</p>		

Project Description:

Objectives

The major objective of this project is to gain a better understanding of how carbohydrates such as glucose and deoxyglucose are transported in glia and neurons and to compare how they are metabolized by these cells. The kinetics of uptake and release of glucose and deoxyglucose will be examined in several types of neurons and glia. Inasmuch as some free deoxyglucose is slowly formed from DG-6-P as it enters the endoplasmic reticulum where it is exposed to G-6-Pase, the mechanism by which the phosphorylated substrate enters the endoplasmic reticulum and the mechanism by which the dephosphorylated product leaves the endoplasmic reticulum and its fate will be investigated.

Major Findings

The methods needed by the planned experiments are being established and tested. Microsomes are prepared from rat liver and rat brain by differential centrifugation through 0.25 M sucrose. In the present studies G-6-Pase activity is measured by the release of inorganic phosphate which is determined colorimetrically by the Chen modification of the Fiske Subbarow phosphate assay. Microsomal protein concentrations are measured by the Markwell modification of the Lowry protein assay.

In experiments in which [^{14}C]deoxyglucose is administered to rats, brains are removed by dissection; microsomes are prepared by differential centrifugation and the free deoxyglucose and DG-6-P are extracted from the microsomal suspensions either, by perchloric acid or by an ethanol-water mixture. Deoxyglucose is measured as the extractable radioactivity that is not retained by Dowex-1 formate; the extractable radioactivity that is retained on the columns and the nonextractable radioactivity represent metabolic products of deoxyglucose.

The degree of "intactness" of the microsomal preparations is measured as the difference in G-6-Pase activity in untreated and detergent-treated microsomes (e.g. sodium deoxycholate).

Experiments have been performed to determine the optimal conditions for assaying microsomal G-6-Pase activity. The rate of hydrolysis of G-6-P has been shown to be linear over at least a 15 min. period for both liver and brain microsomes. The effect of pH on G-6-Pase activity has been examined in conjunction with the use of substrates of nonspecific phosphatases and inhibitors of acid and alkaline phosphatases in order to pick a pH for assaying G-6-Pase activity at which nonspecific phosphatase activities are minimal. The optimal concentration of deoxycholate, a detergent used to disrupt the microsomal membrane which isolates the enzyme, G-6-Pase, from its substrate, G-6-P, has also been determined.

Results from a preliminary experiment with rats injected with a pulse of [^{14}C]deoxyglucose suggest that 8-9% of the total radioactivity remaining in microsomes prepared from brains of rats killed 45 and 88 min. after the pulse is in free deoxyglucose. This finding is consistent with the possibility that deoxyglucose is retained in the endoplasmic reticulum after DG-6-P is hydrolyzed.

These preliminary studies are nearing completion and will be followed by other studies in which the microsomes will be used to determine whether there is a delay in releasing the deoxyglucose that results from hydrolysis of DG-6-P by G-6-Pase.

Significance to Biomedical Research and the Program of the Institute:

This project results from a natural evolution of our interest in the deoxyglucose method. The method as described in 1977 assumed the brain to be composed of localized regions of tissue homogeneous with respect to blood flow, rates of transport of [^{14}C]DG and glucose between plasma and tissue; concentrations of [^{14}C]DG, glucose, [^{14}C]DG-6-P, and G-6-P; and the rate of glucose utilization. While at the macroscopic level these assumptions appear to hold, little is known about the actual concentrations of deoxyglucose and glucose in the extracellular and intracellular spaces in brain or in the neuronal and glial cellular elements. More must be known about the kinetics of glucose and deoxyglucose uptake and release at the cellular level in brain before the deoxyglucose method can be applied to determine quantitative values for glucose utilization at the cellular and subcellular levels.

Proposed Course:

Rate constants for uptake and release of deoxyglucose at the cellular level will be determined in several types of neurons and glia grown in cell culture. Brain dialysis will be used to sample deoxyglucose concentrations in the extracellular space in brain in vivo and these will be compared to plasma concentrations of deoxyglucose held at constant concentrations to determine by various computerized fitting procedures the kinetic parameters of carbohydrate transport in and out of cells.

Publications:

None.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00507-06 LCM

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Brain Imaging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. M. Cohen	Chief, CBI	LCM, NIMH
Others:	T. E. Nordahl	NRSA Clinical Fellow	LCM, NIMH
	M. Gross	Senior Staff Fellow	LCM, NIMH
	A. J. Zametkin	Staff Psychiatrist	LCM, NIMH
	W. Semple	Psychologist	LCM, NIMH
	J. Cappelletti	Computer Programmer	LCM, NIMH
	A. C. King	Psychology Technician	LCM, NIMH

COOPERATING UNITS (if any)

Clinical Neuroscience Branch, Biological Psychiatry Branch, Child Psychiatry Branch, Lab. of Psychology & Psychopathology, NIMH: Clinical Center, NIH

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Clinical Brain Imaging

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

8.5

PROFESSIONAL:

4.5

OTHER:

4.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The major areas of effort in this project have been (1) to develop new tracers or other approaches for the study of neurotransmitter function in normal and abnormal physiology; and (2) to apply available tracer methodologies to the study of neuropsychiatric disorders. To these ends the following achievements are notable.

[18F]-cyclofoxy, an opiate receptor dependent tracer, has been administered to man for the first time. Our initial PET (positron emission tomography) findings are that cyclofoxy accumulation is highest in the amygdala, thalamus, caudate and putamen but also apparent in the cingulate, anterior frontal cortex, and cerebellum. Compared to blood flow and glucose metabolic images, visualization of the amygdala is the unique feature of cyclofoxy studies.

PET measurements of glucose metabolism are increasing our understanding of the functional activities of the brain in psychiatric disorders. In schizophrenia, the data generated support functional abnormalities of the superior parietal and mid-prefrontal cortices, regions to which the ability to sustain attention is localized to in normals. Part of the functional abnormality in schizophrenia appears to be sensitive to neuroleptic treatment. Furthermore, we observe a similar abnormality in manic-depressive disorder, but not in attention deficit, panic and obsessive compulsive disorders. In contrast, we have observed an abnormally high metabolic rate in the orbital frontal cortex of obsessive compulsive patients. Thus, the mid-prefrontal cortex abnormality may reflect a vulnerability factor in the development of psychosis.

OTHER PROFESSIONAL PERSONNEL:

Steven M. Larson, M.D., NM, CC, NIH
 David Pickar, M.D., NSB, NIMH
 Michael Channing, NM, CC
 Richard Carson, Ph.D., NM, CC, NIH

Major FindingsMethod Development:

In the last annual report, we summarized our early findings with a new selective opiate receptor dependent tracer [^{18}F]cyclofoxy (CFY) in which we demonstrated the displacement of cyclofoxy binding with the selective opiate receptor antagonist, naloxone, in baboons. This data is currently being analyzed by two different compartment models to determine a best-fit for the data. We are also determining if a slower infusion of cyclofoxy, although yielding less contrast, will induce a faster equilibrium between brain tissue containing opiate receptors and plasma. Safe production of cyclofoxy for administration to humans has been ensured, and an application for its use in normals and neuropsychiatric patients has been obtained from the Food and Drug Administration. We have successfully administered CFY to normal volunteers with the following results: Tissue radioactivity in the brain came into apparent equilibrium with corrected plasma radioactivity, 45-60 min after injection. A two-compartment model was applied to the data and the volume of distribution, i.e. the ratio of specific plus non-specific binding to plasma, was calculated for each of 10 brain regions. These numbers (means \pm s.d) which should reflect the opiate receptor "avidity," i.e. both the total number of unoccupied opiate receptors and their respective binding affinities for CFY, were in descending order, amygdala, 16.0 ± 7.1 ; thalamus, 15.2 ± 4.0 ; putamen, 12.7 ± 1.3 ; caudate, 11.8 ± 2.5 ; anterior cingulate, 10.3 ± 2.4 ; anterior frontal cortex, 10.1 ± 2.3 ; temporal lobe, 9.24 ± 2.5 ; cerebellum, 9.22 ± 1.2 ; precuneus, 7.60 ± 1.7 ; primary visual cortex, 5.33 ± 1.1 . Compared to blood flow and glucose metabolic images, visualization of the amygdala is the unique feature of cyclofoxy studies (Cohen, et al., Abstract, Society of Nuclear Medicine, June, 1988).

Patient Studies with PET

Brain function has been examined in schizophrenia and other psychiatric disorders in the context of a specific executive function, maintenance of directed attention. A continuous performance test (CPT), based on auditory discrimination, was developed for this purpose because CPTs reliably demonstrate deficits in the maintenance of directed attention in schizophrenia (and other psychiatric disorders) and are presumed to be associated more directly to genetic errors than the overt symptomatology of schizophrenia. Detailed anatomical examination of the frontal cortex is required to localize the ability to sustain attention in man because of the

number of functionally somewhat independent entities which comprise the frontal cortex.

We found that we could successfully apply the FDG-PET methodology to delineate biological determinants of attention, i.e. those anatomical structures that may contribute to the reception and modulation of sensory stimuli. We observed metabolic rate differences in the middle prefrontal, cingulate and superior posterior parietal cortices of normals performing auditory discrimination compared to resting subjects or subjects receiving electric shocks. Furthermore, a direct relationship was observed between metabolic rates in the middle prefrontal cortex and the accuracy of a normal subject's auditory discrimination. We believe that this is the first time that the metabolic activity of a brain region has been specifically linked to quantitative measures of the accuracy of ongoing performance in normals.

As previously reported in patients with schizophrenia, even those who performed as well as normals, the metabolic rate of the middle prefrontal cortex was found to be significantly lower than normal and unrelated to performance. Furthermore, we have partially completed our analysis of the data of 8 patients who were receiving neuroleptics and find that the medicated patients with schizophrenia demonstrate a clear association between brain activity in the mid-prefrontal cortex and performance. The findings point to a role of the mid-prefrontal cortex and its dopamine neurotransmitter pathway input in sustained attention and to dysfunction of this region and of its dopamine modulation in some patients with schizophrenia. Although our analysis is preliminary, abnormalities in similar regions may be present in severe manic-depressive illness. However, this does not appear to be the case with attention-deficit disorder, anxiety disorder, or obsessive compulsive disorder.

In obsessive compulsive disorder we have observed an increase in the metabolic rate of the orbito-frontal region. This study follows an earlier report by investigators from UCLA, but improves on the earlier study by the use of a higher resolution scanner, and by stricter inclusion and exclusion criteria which allowed for the study of obsessive-compulsive illness without contamination with depression. Based on animal studies and our own studies of normal controls receiving mildly painful electric shock, we believe that the orbito-frontal region may be a biological determinant of habituation, extinction, or inhibition of response.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

The central tenet of the project is the belief that our increasing knowledge of the molecular genetics, biochemistry and cytology of the central nervous system will fall short of allowing us a complete understanding of normal and abnormal behavior regardless of the degree of sophistication that genetic probes and postmortem analyses achieve without studies of the functioning system. Advances in the understanding of the functioning system

are required if we are to delineate the pathophysiology of psychiatric illnesses such as schizophrenia. Moreover, such work should facilitate a reduction in the heterogeneity of patient samples in research studies of psychiatric disorders by ensuring that patients who have the syndrome of schizophrenia also share the same pathophysiology. This may be of particular importance to genetic studies.

Further, were a single gene found to be principally responsible for the genetic determination of schizophrenia prior to the elucidation of the pathophysiology, schizophrenia researchers would still need information about pathophysiology to tackle the difficult problems of determining the mechanisms responsible for the development of the phenotype and to improve treatment strategies.

We continue to use positron emission tomography (PET) to elucidate the pathophysiology of psychiatric disorders. PET, one of a number of new brain imaging technologies with its unsurpassed ability to precisely localize and quantify in the human brain tracers used for study of physiological processes, offers the greatest promise for delineating the pathophysiology of schizophrenia and other psychotic disorders.

In our most recent PET studies we chose to examine the functional localization of the ability to perform continuous auditory discrimination because consistent defects in continuous performance had been reported in schizophrenia and in subjects at increased risk for schizophrenia and because sustained attention is also fundamental to the development and execution of all "goal-directed" behavior. These defects may lie closer to the primary defects presumed to be associated with genetic errors than the overt symptomatology of schizophrenia (fully expressed phenotype). We were able to observe dysfunction of the middle prefrontal cortex with respect to sustained attention in schizophrenia. Although the importance of this dysfunction for understanding the pathophysiology of schizophrenia remains to be delineated, preliminary evidence of the dopamine dependence of this function in schizophrenia as evidenced by change in prefrontal cortex function with neuroleptic treatment suggests that detailed studies of anatomic and neurotransmitter pathways involved in attention are warranted.

Moreover, the observation that the two psychiatric disorders, manic-depressive illness and schizophrenia, may share a similar pathophysiology, but that other psychiatric disorders do not, suggest that we may be studying a vulnerability factor in the development of psychosis.

Of equal importance are our findings of dysfunction in the orbito-frontal region in obsessive-compulsive disorder. First, it replicates findings obtained at an independent PET center, UCLA, with an independently obtained sample of patients. It is the exception in psychiatric studies to have such close agreement between two clinical studies with small subject numbers. Further, it extends that work to suggest that this abnormality is related to

obsessive-compulsive disorder independent of depressive symptoms. These observations will lead investigators to study this region with respect to its function in habituation responses, emotion, and the neurotransmitter dependence of this function.

PROPOSED COURSE:

The majority of subjects that have participated in our protocols to date were scanned on the ECAT II scanner. The resolution of the ECAT II scanner was 1.8 cm and the number of slices that could be obtained was, at best, 7. Furthermore, the attenuation corrections were calculated and could not be determined empirically, thus limiting the brain structures that could be accurately examined. The Scanditronix scanner now in use for this protocol has a 5-6 mm resolution; we can gather 28 slices from a single scan, and an empirically derived attenuation correction can be made. Thus, work with the Scanditronix should allow us to replicate our previous findings, search for additional biological determinants of attention and for abnormalities in schizophrenia. The use of other populations, e.g., neuropsychiatric disorders including adult attention deficit disorder (ADD) and the examination of patients while on medications for their disorders (e.g. while ADD patients are on stimulants) should help with the search for additional biological determinants of sustained attention and the delineation of the specificity of these findings for schizophrenia. Our preliminary results in this regard are encouraging in that only the two psychiatric illnesses, manic-depressive illness and schizophrenia, comprising the major psychoses appear to share a similar pathophysiology.

Although our success in pursuing an understanding of the brain metabolic map of sustained attention and a component of its neurotransmitter dependence is gratifying, it is likely that the study of other cognitive processes will be required to define the pathophysiology of an illness as complex as schizophrenia. Just as no one phenomenological variable such as hallucinations is sufficient to delineate schizophrenia, we will probably need to evaluate brain activity with respect to a number of cognitive tasks as well as with respect to the other major component of behavior, emotion, if we are to arrive at an understanding of complex neuropsychiatric disorders such as schizophrenia. Therefore, we will also be pursuing biological determinants of other components of cognition and emotion.

PUBLICATIONS:

Cohen RM, Nordahl TE. Brain Imaging Techniques. In: Howells FG ed. Modern perspectives in clinical psychiatry, New York: Bruner/Mazel, in press.

Cohen RM, Semple WE, Gross M, Nordahl TE. From syndrome to illness: delineating the pathophysiology of schizophrenia with positron emission tomography, Schizophr Bull, in press.

Cohen RM, Semple WE, Gross M, Holcomb HH, Dowling SM, Nordahl TE. Functional localization of sustained attention: comparison to sensory stimulation in the absence of instruction, Neuropsychiatry, Neuropsychology, and Behav Neurol, in press.

Nordahl TE, Benkelfat C, Semple WE, Gross M, King AC, Cohen RM. Cerebral glucose metabolic rates in obsessive compulsive disorder, Neuropsychopharmacol, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02296-03 LCM

PERIOD COVERED
October 1, 1987 to September 30, 1988TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
In Vivo Tomographic Imaging of Dopaminergic Systems and their Turnover

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

C. C. Chiueh Pharmacologist LCM NIMH

COOPERATING UNITS (if any)

Nuclear Medicine, CC, NIH (R. Finn; M. Green); Laboratory of Chemistry, NIDDK (K. Kirk); University of Pennsylvania (H. Kung)

LAB/BRANCH
Laboratory of Cerebral MetabolismSECTION
Section on Clinical Brain ImagingINSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 2.5	OTHER: 0.5
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CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The PET/[F-18]-L-6-F-dopa imaging procedures of brain dopaminergic neurons, including a cyclotron production of radioactive nuclide, radiochemical synthesis and purification of [F-18]-L-6-F-dopa, quality control of radiopharmacy, PET tomographic imaging, and the plasma metabolic profile of the imaging ligand has been developed and tested by this pre-clinical study. The results of the quality control of [F-18]-L-6-F-dopa revealed the chemical purity and the specific activity were greater than 95% and 350 mCi/mmol, respectively. This highly purified [F-18]-L-6-F-dopa preferentially imaged dopamine neurons in the brain in vivo and the imaging ratio of dopamine in the basal ganglia over that of background was improved from 1.5 to 3. Whereas, 2-F-dopa, a by-product of the fluorination procedure, produced no dopamine imaging. The results of the present study of PET/6-F-dopa imaging procedures for brain dopamine in conjunction with the MPTP-induced hemi-parkinsonian primate model have confirmed its potential clinical uses in determining degree of brain damage in parkinsonism and in measuring turnover rate of dopamine neurons in mental disorders. We have also developed a new SPECT imaging ligand, [I-123]-labeled IBZM, for studying D2 dopamine receptors. The in vitro membrane binding study indicated that the binding of IBZM was highly selective to D2 dopamine receptors which was displaceable by both agonists and antagonists of the D2 dopamine receptor. The imaging of the D2 dopamine receptors in the A9, A10, and A16 dopaminergic systems were obtained in vivo within thirty minutes following the administration of the radioactively labeled IBZM by either SPECT or autoradiographic procedures. Thus, the results of this pre-clinical study have provided potential clinical brain imaging ligands for studying pre- and post-synaptic dopaminergic activities in patients with neuropsychiatric or neurological disorders.

Other Professional Personnel Engaged on the Research Project:

R. M. Cohen	Section Chief	LCM		NIMH
D. Doudet	Visiting Fellow	LCM		NIMH
T. Bruecke	Guest	LCM		NIMH
	Researcher			
J. J. Chen	Visiting Fellow	LCM		NIMH
H. Miyake	Visiting Fellow	LCM		NIMH
R. Finn	Cyclotron	NM	CC	NIH
	Director			
M. Green	SPECT Director	NM	CC	NIH
K. L. Kirk	Section Chief	LC		NIADDK
D. Furlano	Chemist			FDA
H. F. Kung	Professor			Dept. of Nuclear Medicine Univ. of Pennsylvania
C. J. Sun	Pharmacologist			FDA

Project Description:**Objectives:**

The first goal of this project is to develop an ideal positron emitting pre-synaptic ligand (either carbon-11 or fluorine-18) for in vivo imaging of brain dopaminergic systems and to provide an index of the functional turnover rate of dopamine by positron emission tomographic (PET) scanning procedures.

The second goal is to develop and evaluate single photon (gamma ray) emitting post-synaptic ligands (iodine-123 labeled compounds: IBZM and IBZP) for imaging of D2 and D1 dopamine receptors in the brain by single photon emission computerized tomographic (SPECT) procedures.

Such tomographic brain imaging procedures may prove to be useful for determining brain damage in Parkinson's disease, for visualizing regeneration of striatal dopamine and for evaluation of up and down regulation of dopaminergic activities and receptors (dopaminergic mechanism) in neuro-psychiatric disorders.

Methods Employed:**A. Synthesis of fluorine-18 labeled L-6-fluoro-dihydroxyphenylalanine (L-6-F-dopa) and iodine-123 labeled 3-iodobenzylamine (IBZM):**

Fluorine-18 labeled L-6-F-dopa is synthesized by Dr. R. Finn using the procedure of Firnau et al. (1984) and Adams et al. (1986). The L-6-F-dopa is separated from 5-F- and/or 2-F-species by using HPLC procedures. [F-18] isotope (2 hr. half-life) is being generated and produced by using the NIH cyclotron in the Nuclear Medicine Department. Carbon-14 labeled 6-F-dopa is enzymatically synthesized by Drs. Furlano and Kirk.

Iodine-123 or iodine-125 labeled 3-iodobenzylamine (specific activity: greater than 1000 Ci/mmol) is synthesized and purified by Kung *et al.* (1987).

B. Animals:

Adult rhesus monkeys (*Macaca mulatta*) of both sexes (5-8 kg) were used. These animals were housed individually in primate quarters on a 12-hr light/dark cycle. Purina monkey chow, water, juice, and fresh fruit were given *ad lib*. L-dopa therapy (Sinemet 100/10, q.i.d.) was given to severely affected parkinsonian monkeys. The hemi-parkinsonism was induced in monkeys after an intra-arterial administration of a dopamine neurotoxin, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine).

During the PET procedures, animals were anesthetized with pentobarbital sodium (35 mg/kg, i.v.). A head mold was used to hold the head steady in the PET scanner. Acute arterial and venous catheters were implanted in order to monitor blood pressure and for blood sampling. Vital signs were monitored throughout the experimental period.

Rats (200 g) or mice (25 g) were pretreated with peripheral decarboxylase inhibitor (MK-486, 25 to 75 mg/kg i.p., 30-60 min.). Carbon-14 labeled L-dopa (sp. act. 10.9 mCi/mmol) was administered through a venous catheter placed in the tail vein. Animals were sacrificed at various times after the treatment for autoradiographic procedures and/or neurochemical assays.

C. Quality Control and Neurochemical Procedures:

The metabolites of 6-[F-18]-dopa in plasma and/or brain samples, i.e., 6-F-dopamine, 6-F-dihydroxyphenylacetic acid, 6-F-homovanillic acid and O-methylated 6-F-dopa, were separated and quantified by HPLC procedures. A semi-preparatory HPLC system was used for an isolation of radioactively labeled 6-F-dopa. These procedures measuring radiochemical purity and specific activity have also been modified for quality control monitoring of the PET imaging ligand.

D. PET Imaging of Brain Dopamine Neurons:

The brain dopamine imaging procedure described by Garnett *et al.* (1983) was used. Briefly, following an intravenous injection of 2 to 4 mCi of purified L-6-[F-18]-dopa, anesthetized monkeys were examined in a 10 mm horizontal brain slice along the orbitomeatal plane in the Scanditronix PET scanner for 2 to 4 hours. At various intervals, blood samples were drawn and assayed for 3-O-methyl-6-F-dopa, the major metabolite of 6-F-dopa. The first 10 min plasma curve of 6-F-dopa was collected for calculation of brain uptake index. In some experiments, [O-15]-water (20 Ci) was used to measure cerebral blood flow in the hemi-parkinsonian monkeys.

E. Autoradiographic Imaging of Brain Dopamine and its Receptors in Small Experimental Animals:

Cerebral *ex vivo* imaging of small experimental animals following intravenous administration of L-3-[C-14]-dopa, L-2-[C-14]-6-F-dopa and [I-125]-IBZM is important for the experimental design of future clinical brain imaging protocols. Mice or unilaterally-lesioned rats were pretreated with antipsychotic agents (haloperidol, YM-09151-2; IBZM), MAO inhibitor (deprenyl), dopamine

uptake blocker (amfonelic acid; GBR-12909), L-dopa decarboxylase inhibitor (NSD-1015), and major tranquilizer (reserpine) in order to manipulate dopaminergic activities in the brain. Animals were sacrificed at various intervals after the administration of imaging ligand in order to simulate clinical brain imaging procedures for investigating the turnover rate of dopaminergic neurons. The brain was quickly dissected and frozen in -20°C isopentane. Brains were cut frozen into 30 μm thick sections and mounted on gelatin-coated slides. Sets of serial sections through the striatum, hypothalamus and mid-brain were processed for autoradiographic demonstration of brain dopaminergic systems using LKB Ultrofilm. The autoradiographic imaging was quantified by using a computerized densitometer and external radioactivity standards.

F. Imaging of D2 Dopamine Receptors by SPECT Procedures:

Radioactive iodo-amphetamine has been used in SPECT procedures for in vivo imaging of blood flow of the human brain. This clinical procedure was modified by Dr. M. Green for the current pre-clinical SPECT/IBZM study using anesthetized subhuman primates.

Major Findings:

We had established a neurochemical basis for the use of purified 6-F-dopa as a presynaptic imaging ligand for brain dopamine. Its application for clinical brain imaging studies is being adapted and tested pre-clinically in collaboration with the PET core program of the Department of Nuclear Medicine of the NIH Clinical Center. We have established a quality control procedure for the chemical and radioactive purity of the fluorodopa imaging ligand, [F-18]-6F-dopa synthesized by Dr. R. Finn. Quality control studies revealed a major contaminant of the 6-F-dopa, 2-F-dopa. Our subsequent in vivo study indicated that 2-F-dopa, although slowly decarboxylated to form 2-F-dopamine, is rapidly O-methylated to 3-methoxy-2-F-dopa and thus interferes with the 6-F-dopa imaging of brain dopamine neurons. Thus, [F-18] labeled 2-F-dopa does not yield PET imaging of brain dopamine. This result is consistent with our previous hypothesis that only 6-F-dopa, and not 2-F- or 5-F-dopa, is useful in PET imaging of brain dopaminergic neurons (Chiueh et al., 1984). The requirement of a highly purified [F-18] labeled 6-F-dopa in future clinical studies is obvious. Dr. R. Finn, Director of the NIH cyclotron facility, and his group have improved the purification procedure of 6-F-dopa and provided a 95% pure imaging ligand for the present pre-clinical study.

Highly purified 6-[F-18]-dopa (>95% purity, 300 mCi/mmol, 2 to 5 mCi i.v.) produces a high contrast image of brain dopamine in the basal ganglia of anesthetized monkeys as visualized by the Scanditronix PET scanner. Two to four hours following the injection of purified ligand, the ratio of striatal to cerebellar [F-18]-dopamine activity reaches 3. This may be due to a consequence of little formation and/or accumulation of 3-O-methyl-6-F-dopa in the plasma as revealed by a HPLC assay of the plasma samples. This imaging ratio was decreased in MPTP treated monkeys and was dropped to one (background) in severely lesioned parkinsonian monkeys. The turnover rate of newly synthesized striatal 6-F-dopamine in the control was slow with a half-life of about four hours. Similar to our postmortem study (Chiueh, 1988), the current PET procedure revealed in vivo an increase in the dopamine turn-

over in mildly affected parkinsonian monkeys. The results of the present PET study (14 scans) at NIH confirm the results of our preliminary study of MPTP-lesioned monkeys using a purified [F-18]-L-6-F-dopa at McMaster Medical Centre (Chiueh et al., 1986). In conclusion, only purified 6-[F-18]-dopa provides specific PET imaging of brain dopamine as reported previously by Garnett et al. (1983). The preferential labeling of dopaminergic neurons by 6-F-dopa might be due to a high dopa decarboxylase activity and a huge accumulation of newly synthesized 6-F-dopamine in storage granules found in dopaminergic terminals. This pre-clinical study suggests that PET/6-[F-18]-dopa procedures can be used not only for studying brain damage in Parkinson disease but also for assessing dopamine turnover in the brain of mental disorder (Chiueh et al., 1988). These results will be reported at the International Symposium of Nuclear Medicine (October, 1988).

In addition to the PET/6-F-dopa study, we have also employed SPECT and autoradiographic imaging procedures for developing of other imaging ligands. It is currently believed that MPTP exerts its toxic effects through its metabolite MPP⁺. We reported that intranigral injection of MPP⁺ caused a dose dependent depletion of striatal dopamine (Namura et al., 1987) and a release of monoamines in rats. In the present study (Sun et al., 1988; Chiueh et al., 1988), we used radioactive pre- and post-synaptic ligands in addition to [Ca-45] to visualize MPP⁺ effects on dopaminergic systems. Autoradiographic procedures were performed two weeks after a unilateral lesioning of the median forebrain bundle following intravenous administration of [C-14]-L-dopa or [I-123]-IBZM. In controls, the in vivo presynaptic [C-14]-L-dopa imaging revealed dopamine-rich areas, such as the caudate nucleus, the nucleus accumbens, and the median eminence. Striatal dopamine fiber imaging completely disappeared in the striatum of the unilaterally nigral-lesioned rats. A denervation-induced dopamine receptor supersensitivity was visualized by using in vivo [I-125]-IBZM binding and ex vivo autoradiographic procedures (in collaboration with Dr. Kung of the University of Pennsylvania). D2 dopamine receptors as seen by the [I-125]-IBZM binding increased by 50% in the denervated side of basal ganglia of unilateral-lesioned rats and in hemi-parkinsonian monkeys. The S(-)-[I-125]-IBZM binding to brain receptors was found to be highly specific to D2 dopamine receptor sites and displaceable by both D2 agonists and antagonists. [I-125]-IBZM bond to other receptors; i.e., D-1, S-2, α -1, and α -2, with very low affinity (Bruecke et al., 1988). Furthermore, the in vivo imaging of D2 dopamine receptors in the brain, obtained within thirty minutes following the administration of [I-125]-IBZM, yielded a four to one ratio in specific to nonspecific activity (Singhaniyom et al., 1988). We are currently testing the use of IBZM labeled with a short half-life (13 hours) isotope [I-123] in SPECT imaging procedures (Dr. M. Green) for D2 dopamine receptors in the brain of living primates.

Significance to Biomedical Research:

The MPTP-induced primate model of parkinsonism was employed in the present pre-clinical study for the trial of these newly developed PET and/or SPECT imaging tracers and for assessment of brain dopaminergic functions in the living organism. The current pre-clinical results show clearly that [F-18]-6-F-dopa and [I-123]-IBZM are excellent dopamine imaging ligands and have potential uses as diagnostic tools in the clinic for identifying parkinsonian patients even in the subclinical stage and for elucidating dopamine mechanism

of neuropsychiatric disorders. Further development could lead to their use in the investigation of brain dopamine turnover rate and D2 dopamine receptor density in neuropsychiatric disease.

Proposed Course:

A. Pre-clinical Studies:

The 6-F-dopa/PET procedures including the generation of [F-18] gas by a cyclotron, the fluorination of L-dopa, purification of [F-18]-labeled 6-F-dopa, and PET scanning of living monkeys have been successfully tested at the NIH medical center. For the application of 6-F-dopa/PET brain imaging procedures to human subjects, Dr. Finn has established a vigorous HPLC purification procedure and we have inaugurated a quality control HPLC procedure for determining the chemical purity and specific activity of each batch of the imaging ligand. However, additional radiation safety (dosimetry) and toxicological evaluations have to be performed by using the Posicam whole body imaging system in order to meet the safety guidelines stipulated by the Food and Drug Administration.

1. Turnover of Brain Dopaminergic Systems Following Administration of Antidepressants, Antipsychotics, Tranquilizers, and Antiparkinsonian Drugs:

Efforts will be focused on the PET procedure for measuring the in vivo turnover rate of dopamine in the mesolimbic and mesocortical systems. The previously published procedures of measuring turnover rate of brain dopamine required at least 5-6 animals per group at 3 different time points. The present PET procedure by using a purified [F-18]-6-F-dopa (> 95% purity) can offer a complete time curve in each animal in vivo following neuropharmacological manipulations. Some of this PET study will be simulated in small animals by using [C-14] labeled 6-F-dopa in conjunction with the brain dialysis perfusion procedure.

- a. Dopamine receptor antagonists: Antipsychotics; IBZM; YM-09151-2
- b. Dopamine uptake blockers and dopamine releasing agents: MPP⁺, d-amphetamine, and cocaine
- c. Electrical activation of the dopaminergic systems: e.g., electrical convulsion shock treatment
- d. MAO inhibitors: antidepressants

2. In vivo SPECT Imaging of D2 Dopamine Receptors by [I-123]-IBZM:

The newly developed D2 dopamine receptor imaging ligand will be tested in clinical SPECT imaging camera by using hemi-parkinsonian monkey model. Our in vitro autoradiographic study has demonstrated a 25 to 50% increase in the binding sites of D2 dopamine receptors in the caudate nucleus and putamen of the MPTP-lesioned parkinsonian monkeys. SPECT procedures will be modified and employed in conjunction with [I-123]-labeled IBZM with a 13-hour half-life. This SPECT imaging ligand is synthesized and provided by Dr. H. Kung of the University of Pennsylvania.

3. Brain Imaging Ligands for Dopamine Uptake Sites:

The chemical structure of GBR-12909, one of the most selective dopamine uptake blockers, will be labeled with a short half-life nuclide, such as [F-18], [I-123], or [C-11], and tested for its usefulness in in vivo PET and/or SPECT imaging procedures for dopamine neurons in the brain.

B. Clinical Studies:

After passing the pre-clinical toxicological evaluations, these pre- and post-synaptic dopaminergic ligands will be administered to normal volunteers, parkinsonian patients, bipolar manic-depressive patients, schizophrenic patients, and other patients suffering from disorders which may involve brain dopamine. These clinical projects will be conducted following established NIH guidelines and with the collaboration of clinical branches of NIMH and NIH.

Publications:

Bruecke T, Tsai YF, McLellan C, Singhaniyom W, Kung HF, Cohen RM, Chiueh CC. In vitro binding properties of the [I-125] labeled benzamide (IBZM): a potential imaging ligand for D₂ receptor in SPECT, Life Sci 1988; 42: 2097-2104.

Chiueh CC. Dopamine in the extrapyramidal motor function: a study based upon the MPTP-induced primate model of parkinsonism. In: Joseph J, ed. Central determinants of aged-related decline in motor function. New York: Annals New York Academy of Science, 1987; 515: 226-238.

Chiueh CC, Cohen RM, Kirk KL, Finn RD, Firnau G, Kung HF. Imaging of pre- and post-synaptic adaptations in the nigrostriatal dopaminergic system of MPTP-induced parkinsonism. In: Beart PM, Jackson DM, eds. Dopamine systems and their regulation. London: MacMillan Press, 1988; in press.

Singhaniyom W, Tsai YF, Bruecke T, McLellan C, Cohen RM, Kung HF, Chiueh CC. Blockade of in vivo binding of [I-125]-labeled 3-iodobenzamide (IBZM) to dopamine receptors by D-2 antagonist and agonist. Brain Res 1988; 453: 393-6.

Sun CJ, Johannessen JN, Gessner W, Namura I, Singhaniyom W, Brossi A, Chiueh CC. Neurotoxic damage to the nigrostriatal system in rats following intranigral administration of MPDP⁺ and MPP⁺. J Neural Transmission 1988; in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00931-15 LGCB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Characteristics and Regulation of S-Adenosylhomocysteine Hydrolase

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	P.S. Backlund, Jr.	Research Chemist	LGCB NIMH
	R.R. Aksamit	Research Chemist	LGCB NIMH
	G.L. Cantoni	Chief, Laboratory of General and Comparative Biochemistry	LGCB NIMH

COOPERATING UNITS (If any)

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SECTION

Section on Proteins

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS

4.5

PROFESSIONAL:

2.5

OTHER:

2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

S-Adenosylhomocysteine hydrolase plays a critical role in regulating AdoMet-dependent methylations in eukaryotic cells by regulating the ratio of AdoMet/AdoHcy. Several approaches are being used to determine the structure and function of this enzyme.

1) Structure Determination: The enzyme has been cloned from a rat liver and a D. discoideum cDNA library. The amino acid sequence was determined and a putative NAD binding site identified. Comparison of the amino acid sequence of the rat liver with the Dictyostelium enzyme indicates that 74% of the amino acids are identical, demonstrating that the enzyme is highly conserved. The cloned cDNA's have been expressed in E. coli and site-directed mutagenesis of the rat liver enzyme is in progress to determine the function of specific amino acid residues in NAD binding and catalytic activity of the enzyme. The expression of the AdoHcy hydrolase mRNA in different tissues and the genomic organization of the AdoHcy hydrolase gene are under investigation.

2) Biological Effects: A large number of adenosine and adenosylhomocysteine analogs have been examined for their ability to function as inhibitors and/or substrates of S-adenosylhomocysteine hydrolase. In vivo these adenosine analogs can form very potent and specific inhibitors of transmethylation reactions, and these inhibitors have a wide range of biological activities, including antiviral activity against several RNA and DNA viruses, inhibition of leukocyte chemotaxis, and stimulation of cell differentiation.

Other Investigators:

J. Kasir	Visiting Fellow	LGCB NIMH
T. Caryk	Chemist	LGCB NIMH
M. Fujioka	Toyama Medical & Pharmaceutical University, Toyama, Japan	

Project Description:

As is well known, S-adenosylmethionine (AdoMet) is a key intermediate in biological transmethylation and transalkylation reactions. There are hundreds of reactions, each catalyzed by a specific enzyme, that utilize AdoMet as a substrate. It is obvious that the utilization of AdoMet in biological systems must be under regulatory controls, but at the present time little is known about the nature of these controls. It has been established that S-adenosylhomocysteine (AdoHcy), one of the products of transmethylation reactions that utilize AdoMet as methyl donor, is a competitive inhibitor of most reactions in which AdoMet participates. From the result of work in this and other laboratories, it has been proposed that the intracellular ratio of AdoMet/AdoHcy must be of key importance in the regulation of biological alkylation reactions, and that this ratio plays a role in determining the hierarchy of biological methylation reactions. In eukaryotes, AdoHcy is metabolized through a single metabolic pathway by S-adenosylhomocysteine hydrolase (AdoHcyase), an enzyme which catalyzes the reversible hydrolysis of AdoHcy to adenosine and homocysteine. Because of the central role of AdoHcyase in the metabolism of AdoHcy and in maintaining the ratio of AdoMet/AdoHcy, this enzyme has been under intensive study in this and other laboratories.

S-Adenosylhomocysteine hydrolase has been purified from a variety of sources. Previous work has shown that the mammalian enzyme consists of structurally identical subunits, contains four mols of tightly bound NAD/mol of enzyme, and also binds cAMP and adenosine. The chemistry of the catalytic reaction is fairly well understood, but very little is known about the structure of the enzyme and its relation to function. Our studies are directed towards 1) the elucidation of the primary structure of the hydrolase by molecular cloning of its cDNA and by inference, its secondary and tertiary structure, 2) the determination of the specific polypeptide sequences that are involved in its binding, catalytic, and regulatory sites, 3) characterization of the conformational changes that accompany activation and binding of substrates and cofactors, and 4) crystallization of the enzyme to provide an absolute three-dimensional structure by X-ray diffraction.

The enzyme was cloned from a rat liver cDNA library, using antibodies directed against the purified enzyme to screen a gt11 cDNA library. The cloned cDNA sequence was then used to screen a cDNA library from Dictyostelium discoideum, in order to clone the Dictyostelium enzyme. The amino acid sequence of the protein was determined, and a putative NAD binding region was identified by the homology of the amino acid sequence with other NAD binding proteins. Comparison of the amino acid sequence of the rat AdoHcy hydrolase with the D. discoideum enzyme indicated that the sequences are highly conserved. When the two sequences are aligned, 74% of the amino acids are identical, and if conservative changes are taken into account, the homology is 84%. The differences in amino acids between the rat and Dictyostelium enzymes appear to occur randomly

throughout the sequence. In light of the large evolutionary difference between rat and Dictyostelium, the striking homology in the AdoHcy hydrolase from these two species suggests that the conservation of amino acid structure is required for the function of the enzyme. The cloned hydrolase was also expressed in E. coli, at an induced level reaching approximately 10% of the bacterial proteins. Site directed mutagenesis of the rat enzyme is also in progress, in order to examine structure/function relationships for different regions of the enzyme, such as the NAD and adenosine binding sites. The cloned cDNA sequence is also being used to examine the level of mRNA expression in different cell types, and in other species. In addition, the genomic organization of the hydrolase in rat is also being examined.

The hydrolase has binding affinities for nucleosides and we are investigating the possible role of ATP, adenosine, cAMP, and the tightly bound NAD's in the regulation of the enzyme. We have shown that the enzyme is inactivated by Mg^{++} , ATP, and KCl with the loss of four molecules of NAD, and it can be reactivated upon incubation of the enzyme with NAD. When NAD is bound to the enzyme, little cAMP binds, while more cAMP binds to the enzyme lacking NAD suggesting that the cAMP may bind to the NAD site. ATP, adenosine, and cAMP have binding affinities for the enzyme and it is not clear how they fit in catalysis and/or regulation. Various affinity reagents have been used to label the hydrolase, and the labeled fragments will be isolated and sequenced to determine the amino acid residues that comprise the active site and/or binding clefts in the protein. The amino acid sequence determined from the cloned cDNA sequence will help to determine the regions of the protein modified by these affinity reagents. Modification of the amino acids at these sites by site directed mutagenesis, will provide independent data on the role of these amino acid residues in catalysis.

While the biochemical mechanisms of transmethylation reactions have been elucidated many years ago, largely as a result of the studies by Cantoni and his collaborators at NIH, the correlation between many methylation reactions and cellular functions remains obscure. For instance, the significance of the methylation of a variety of informational macromolecules, such as proteins and nucleic acids (DNA, ribosomal-, messenger-, viral and tRNA, etc.), or of complex polysaccharides, or even simpler compounds such as guanido acetic acid, nicotinamide, etc., is not immediately obvious and is the subject of much debate. A role for DNA methylation in gene expression has been suggested by observations from several laboratories. We have shown that both 3-deaza-Ado and 3-deaza-Ari can stimulate cell differentiation in a number of cell lines. It is possible that 3-deaza-Ado may cause differentiation of these cells by inhibiting DNA methylation. In collaboration with Dr. Razin, we have proposed a novel mechanism for the transient demethylation of DNA during differentiation where 5-methylcytosine is replaced enzymatically by cytosine, by a mechanism distinct from conventional excision-repair (see Z01 MH 02321-2 LGCB).

Since AdoHcyase is the only enzyme known to metabolize AdoHcy in eukaryotes, inhibition of this enzyme by analogs can be used to alter the ratio of AdoMet/AdoHcy in the cell. We decided some years ago to take advantage of this fact and initiated a long range experimental project designed to study in depth the properties of AdoHcyase, and then to develop a series of specific inhibitors of this enzyme. As a result of these studies on the properties of AdoHcyase, we

have established that the use of specific inhibitors makes it possible to alter the intracellular levels of AdoHcy and/or to accumulate intracellularly congeners of AdoHcy of the general formula S-purinyihomocysteine (PurHcy). By using these inhibitors, it is possible to modulate the AdoMet/AdoHcy and/or AdoMet/PurHcy ratio in different cellular systems, and to examine the consequences of these changes on cellular functions.

Our studies, confirmed and extended in other laboratories, have identified several inhibitors of AdoHcyase. These compounds have a variety of biological effects and may have important clinical applications, and explain some of the mechanisms of action of some clinically important compounds. Irreversible inhibitors of AdoHcyase include the compounds 9- β -D-arabinofuranosyladenine (Ara-A), 3-deaza-9- β -D-arabinofuranosyladenine (3-deaza-Ara-A), and 2-chloroadenosine. Ara-A has been used by others in chemotherapy for cancer patients. 3-Deaza-Ara-A and 2-chloroadenosine might be expected to have clinical effects similar to Ara-A, since they produce similar inhibition of AdoHcyase. Of the many reversible inhibitors tested, two compounds have been extensively studied in this laboratory as prototype compounds of this group; 3-deazaadenosine (3-deaza-Ado) and 3-deazaaristeromycin (3-deaza-Ari). 3-Deaza-Ado is a potent competitive inhibitor of AdoHcyase with K_i of 5-8 μ M; and as a substrate has a K_m value about equivalent to the natural substrate, adenosine. In contrast to 3-deaza-Ado, 3-deaza-Ari is not a substrate for AdoHcyase, but it is a very potent competitive inhibitor, with K_i of 2.0 nM for the hamster liver enzyme. Neither compound is a substrate for either adenosine kinase or adenosine deaminase.

The capacity of AdoHcyase to synthesize AdoHcy analogs in vivo, as has been shown with 3-deaza-Ado, demonstrates the exciting possibility of synthesizing potent and specific methylation inhibitors intracellularly. Comparison of the biological effects of 3-deaza-Ado and 3-deaza-Ari has made it possible to attribute some of the differences in specificity to the finding that 3-deaza-AdoHcy is a more potent and specific inhibitor of some transmethylation reactions than AdoHcy. We have found that macrophage chemotaxis is specifically inhibited by the intracellular formation of 3-deaza-AdoHcy, brought about by treatment of the cells with 3-deaza-Ado, while chemotaxis is unaffected by accumulation of AdoHcy by treatment with 3-deaza-Ari (see Z01 MH 00942-07). We have further shown that treatment of cells with 3-deaza-Ado inhibited mRNA synthesis to a much greater extent than treatment with 3-deaza-Ari. Both 3-deaza-Ado and 3-deaza-Ari were used to inhibit mRNA methylation, and the methylation of adenosine on the N-6 position was very sensitive to inhibition, while methylation of the guanosine in the mRNA cap was only slightly inhibited. Both 3-deaza-Ado and 3-deaza-Ari also inhibit the replication of various RNA and DNA viruses. The sensitivity of various viruses to these two drugs is different, and it seems probable the some of the antiviral effects can be attributed to an inhibition of viral mRNA synthesis or methylation. The specific reaction(s) involved in inhibition of RNA synthesis has not been identified, and the effect of both compounds on viral RNA methylation may be useful for examining the role of these reactions in the synthesis and processing of different classes of viral RNA.

Our studies on protein methylation have recently identified a novel class of protein methylation. We have identified a guanine nucleotide-dependent carboxyl methylation of several membrane proteins in mammalian cells. The methylation of

membrane proteins of M_r 20-23K requires AdoMet, GTP or non-hydrolyzable GTP-analogs, and a cytoplasmic methyltransferase. The methylation was shown to be a carboxyl methylation by hydrolysis under basic conditions to produce methanol. However, the base lability of this methylation was much less than expected for aspartyl carboxyl methyl-esters. The role of guanine nucleotide-binding membrane proteins in regulating receptor mediated functions is well documented, and several families of these membrane proteins have been identified. The guanine nucleotide dependence and the physiologically reversible nature of carboxyl methylations suggests that these methylations may regulate the function of some guanine nucleotide-binding membrane proteins.

In a series of recent studies in Europe and in this country, it has been found that AdoMet, given parenterally to depressed patients produced rapid and remarkable improvement in the clinical picture. These studies indicate that AdoMet has approximately the same antidepressant activity as the standard tricyclics, such as imipramine, amitryptiline, etc. It is noteworthy, however, that administration of AdoMet is not accompanied by any toxic side effects, and thus, this mode of therapy may represent a considerable improvement over the therapeutic regimens currently in use. The mechanism of action of Adomet in depressive illness is unknown. It should be pointed out, however, that the dose of AdoMet found to be effective in the management of clinical depression (200-400 mg/i.v./day) is very small compared to the daily flow of methionine through AdoMet. Human adults synthesize and metabolize about 20 millimoles of AdoMet/day, or 20-40 times the dose used in clinical trials.

Significance to Biomedical Research and the Program of the Institute:

Studies of the AdoHcyase and its inhibitors are important to understanding the regulation and function of biochemical transmethylation, and have possible clinical applications in the development of specific inhibitors for certain methylation reactions. Since AdoMet dependent methylation reactions are involved in the synthesis of so many compounds, including DNA, RNA, proteins, lipids, and neurotransmitters, the regulation of these reactions can alter many cell functions. Inhibitors of methylation reactions have been shown to affect cell differentiation, leukocyte chemotaxis, and virus replication. The possible clinical applications could be in the development of compounds for use in chemotherapy, immunosuppression, and antiviral drugs. Because of the important role of methylation in neurotransmitter synthesis, these compounds could have important effects on brain function as well.

Proposed Course of Research:

The cloned cDNA sequence will be used for site directed mutagenesis in order to characterize the role of specific amino acids in the enzyme catalysis. The genomic organization of the hydrolase gene and the tissue specific expression will also be investigated. Studies on several inhibitors will continue in order to determine specific mechanisms of inhibition, and to determine correlations between inhibition of specific reactions and the physiological effects of these compounds. Much of the work will focus on methylation reactions involved in leukocyte chemotaxis, and on the role of DNA methylation in gene expression. Work will continue on the characterization of the guanine nucleotide-dependent carboxyl

methylation of membrane proteins in order to characterize the identity of the methylated proteins and the effect of methylation on their function.

Publication:

Kasir J, Aksamit RR, Backlund PS Jr, Cantoni GL. Amino acid sequence of S-adenosyl-L-homocysteine hydrolase from Dictyostelium Discoideum as deduced from the cDNA sequence, Biochem Biophys Res Commun 1988;in press.

Ogawa H, Date T, Gomi T, Konishi K, Pitot HC, Cantoni GL, Fujioka M. Molecular cloning, sequence analysis, and expression in Escherichia coli of the cDNA for guanidinoacetate methyltransferase from rat liver, Proc Natl Acad Sci USA 1988;85:694-9.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00936-24 LGCB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Homocystinuria: Methionine Metabolism in Mammals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

S.H. Mudd Chief, Section on Alkaloid Biosynthesis LGCB NIMH

COOPERATING UNITS (if any) William Gahl, Human Genetics Branch Child Health and Human Dev.
James Finkelstein, VA Hospital and George Washington Univ., Washington, D.C.
Alfred Tangerman, Dept of Medicine, St. Radboud Univ. Hospital, Nijmegen, The Netherlands

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Alkaloid Biosynthesis

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

0.1

PROFESSIONAL:

0.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Z01 MH 00936-24 LGCB has been terminated because the Section on Alkaloid Biosynthesis has been closed.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00940-07 LGCB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Methionine Biosynthesis in Higher Plants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

A.H. Datko Biologist

LGCB NIMH

S.H. Mudd Chief, Section on Alkaloid Biosynthesis

LGCB NIMH

COOPERATING UNITS (if any)

R. Aksamit, LGCB, NIMH

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TOTAL MAN-YEARS

1.7

PROFESSIONAL:

1.7

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Z01 MH 00940-07 LGCB has been terminated because the Section on Alkaloid Biosynthesis has been closed.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00942-07 LGCB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Biochemical Reactions in Mammalian Cell Chemotaxis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. R.R. Aksamit Research Chemist LGCB NIMH

P.S. Backlund, Jr. Research Chemist LGCB NIMH

G.L. Cantoni Chief, Laboratory of General and Comparative Biochemistry LGCB NIMH

COOPERATING UNITS (if any)

Office of Biologics, FDA; Molecular Pathophysiology Section, NIDDK;
Department of Biochemistry and Pharmacology, University of Glasgow, Scotland

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TOTAL MAN-YEARS:

1

PROFESSIONAL:

1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies on the inhibition of RAW264 macrophage cell line chemotaxis by 3-deazaadenosine have led us to postulate that incubation of cells with 3-deazaadenosine inhibits methylation reaction(s) required for the formation of functional mRNA coding for one or more chemotaxis proteins. Efforts to identify proteins that may play a central role in RAW264 chemotaxis have been limited because chemically defined attractants for RAW264 cells have not been available. This problem was overcome by the isolation of a stable cell hybrid from a fusion between human leukocytes and a thioguanine-resistant RAW264 cell line. The hybrid expressed functional genes for chemotaxis to fMet-leu-phe, a commercially available synthetic attractant. The cell hybrid, granulocytes from several species (mouse, guinea pig, and rabbit) and human monocytes exhibited chemotaxis to fMet-leu-phe and to fMet-leu-phe oxidized to either the sulfoxide or sulfone. In contrast, human neutrophils did not migrate to oxidized fMet-leu-phe. These observations indicated that the human neutrophil may be unique in its lack of chemotactic responsiveness to oxidized fMet-leu-phe, and suggested that the fMet-leu-phe receptor complex or chemotaxis transduction mechanism may be different in human neutrophils than in other phagocytic leukocytes. We have shown that one or more guanine nucleotide binding proteins are required for chemotaxis by RAW264 and the hybrid cells. This conclusion is based on the observation that chemotaxis of either RAW264 or hybrid cells is inhibited upon incubation of the cells with either cholera toxin or pertussis toxin. For both toxins, entry into the cell is required and there is a correlation between toxin-catalyzed ADP-ribosylation of a guanine nucleotide binding protein and the inhibition of chemotaxis. By immunochemical and electrophoretic techniques, the pertussis toxin substrate involved in chemotaxis has been identified as G_i-2, a protein that is also found in brain. In addition, a second family of guanine nucleotide binding proteins of M_r 20-23K has been identified in RAW264 cells. Whether or not this family of proteins is involved in chemotaxis has not yet been determined.

Other Investigators:

A. Spiegel	Chief, Molecular Pathophysiology Section	NIDDK
G. Milligan	Assistant Professor, Univ. of Glasgow	
T.M. Caryk	Chemist	LGCB NIMH
L. Harvath	Research Microbiologist	DBBP FDA

The important discovery in this laboratory that chemotaxis by a macrophage cell line is specifically inhibited by 3-deaza-AdoHcy has allowed us to assess the significance of certain biochemical reactions in macrophage chemotaxis. Our conclusion was based on the finding that RAW264 chemotaxis is inhibited by 3-deazaadenosine but not by 3-deazaaristeromycin, and a search was initiated for a biochemical reaction that also showed this inhibitor specificity.

The synthesis of phosphatidylcholine by methylation of phosphatidylethanolamine, the release of arachidonic acid when cells are incubated with EAMS (endotoxin-activated mouse serum, an attractant for mouse macrophages), methylation of the lysine and arginine residues of protein, and protein carboxymethylation were all inhibited by both 3-deazaadenosine and 3-deazaaristeromycin. From these studies we conclude that none of these reactions are required for chemotaxis by RAW264 cells.

In contrast, the synthesis of a small number of proteins, identified after separation by two-dimensional polyacrylamide gel electrophoresis, does show the necessary inhibitor specificity for involvement in RAW264 chemotaxis. Quantitation of 100 of the more prominent proteins on the gels by computerized densitometry showed that in cells treated with 3-deazaadenosine the synthesis of approximately 10% of the proteins was inhibited by more than 50%, whereas in cells treated with 3-deazaaristeromycin the synthesis of these proteins was not significantly inhibited. The correlation of the inhibition of a subset of proteins with the inhibition of chemotaxis was strengthened by the finding that other inhibitors of chemotaxis inhibited the synthesis of the same subset of proteins. These inhibitors are 3-deoxyadenosine and the combination of erythro-9-(2-hydroxy-3-nonyl)adenosine (EHNA), adenosine and homocysteine. A common feature of the inhibitors of chemotaxis described above is that they all can inhibit the synthesis of functional mRNA. In this regard, we have also found that inhibitors of protein synthesis and translation, such as cycloheximide, puromycin and actinomycin D, inhibit chemotaxis.

We have proposed as a working hypothesis that treatment of RAW264 cells with 3-deazaadenosine, 3'-deoxyadenosine, and the combination of EHNA, adenosine and homocysteine inhibits the synthesis of functional mRNA coding for one or more chemotactic proteins. In support of this hypothesis, we have found that 3-deazaadenosine is a more potent inhibitor of polyadenylated mRNA than 3-deazaaristeromycin and that AdoHcy and 3-deazaAdoHcy do not inhibit in vitro translation.

Time-lapse video cinematography shows that motility and EAMS-induced morphological changes are similar in 3-deazaadenosine-treated and control cells. These observations suggest that in cells treated with 3-deazaadenosine, signal processing after attractant binding to the chemoreceptor is inhibited.

Additional studies to examine directly the effects of 3-deazaadenosine on attractant binding or to investigate the steps in signal transduction have been hindered by the lack of chemically defined attractants. The attractants described for RAW264 cells, EAMS and LDCF (lymphocyte-derived chemotactic factor), are both complex molecular mixtures with multiple biological activities. On the other hand, human monocytes and neutrophils are known to exhibit chemotaxis to FMLP (N-formylmet-leu-phe), a commercially available synthetic attractant. For these reasons hybrid cells were isolated from fusions between human leukocytes and thioguanine-resistant RAW264 cells, and some of the hybrids exhibited chemotaxis to FMLP and structurally related N-formylpeptides. The WBC264-9 cell line has been cultured for more than 6 months without loss of chemotaxis to FMLP demonstrating that a stable cell line has been obtained.

Chemotaxis of WBC264-9 and human leukocytes to FMLP are similar in several respects. The concentrations of N-formylpeptides that elicit the optimal chemotactic response in WBC264-9 cells are similar to the optimal chemotactic concentrations reported for human leukocytes. WBC264-9 migrates more quickly to FMLP than to EAMS, and the time course of WBC264-9 migration to FMLP is similar to that of human leukocytes. It also appears that WBC264-9 chemotaxis to FMLP and to EAMS may be regulated independently.

However, a study of the binding of radiolabeled FMLP to WBC264-9 cells indicated that WBC264-9 cells contained fewer receptors than human leukocytes do, although it should be noted that the receptor number is sufficient for chemotaxis. To reduce the technical problem of nonspecific binding, the number of receptors on membrane preparations was determined. From this data it was calculated that there are approximately 1200 receptors per WBC264-9 cell, compared to reported values from 2000 to 120,000 per cell for human leukocytes. The apparent dissociation constant for FMLP binding to WBC264-9 membranes was 2 nM, in agreement with values reported for the high affinity site of human leukocytes. It has been proposed that the high affinity receptors for FMLP mediate chemotaxis.

Studies on the human FMLP receptor have been carried out in collaboration with Dr. L. Harvath. Our laboratory's principal contribution has been the preparation and analytical determination of chemical derivatives of FMLP. These studies have shown that human monocytes exhibit chemotaxis for both FMLP sulfoxide and sulfone, whereas human neutrophils do not exhibit chemotaxis to either of the oxidized peptides. In contrast, both human neutrophils and monocytes migrate to nonoxidized FMLP, and both cell types generate superoxide anion, secrete enzymes and polarize when stimulated with FMLP, FMLP sulfoxide or FMLP sulfone. These data suggest that the FMLP receptor complex or chemotaxis transduction mechanism is different in human neutrophils than in monocytes. Extension of this study to other species has indicated that the human neutrophil may be unique in its lack of chemotactic responsiveness to oxidized FMLP derivatives. Mouse, guinea pig, and rabbit granulocytes, cells functionally analogous to the human neutrophil, exhibit chemotaxis to oxidized FMLP.

In collaboration with Dr. Harvath, we have also developed flow cytometric procedures that allow us to determine the subpopulation of leukocytes in whole

blood that bind a fluorescent FMLP derivative and to determine the rate of binding of fluorescent FMLP to human neutrophils.

In addition to chemotactic receptors, one or more guanine nucleotide proteins (G-proteins) are required for chemotaxis by RAW264 and WBC264-9 cells. This conclusion is based on the observation that chemotaxis of either RAW264 or WBC264-9 cells is inhibited upon incubation of the cells with either cholera toxin or pertussis toxin. In all cases entry of the toxin is required and there is a correlation between toxin-catalyzed ADP-ribosylation of membrane protein and the inhibition of chemotaxis.

Although both cholera toxin and pertussis toxin can affect cAMP levels, our evidence indicates that cAMP is not involved in chemotaxis. This was shown by elevating cAMP with either isoproterenol or forskolin to levels comparable to those achieved with cholera toxin. Chemotaxis of cells treated with isoproterenol or forskolin was not inhibited, showing that increased levels of cAMP per se do not inhibit chemotaxis.

In agreement with observations in several other laboratories, we found that the major membrane protein ADP-ribosylated by cholera toxin is distinct from that ADP-ribosylated by pertussis toxin. This was shown by the different electrophoretic mobilities of the proteins and the difference in the nucleotide specificity of the ADP-ribosylation reactions. However, under certain conditions, cholera toxin also appeared to ADP-ribosylate a membrane protein with a molecular weight similar or identical to the substrate for pertussis toxin.

Further immunochemical studies that employed a battery of antisera specific for several pertussis toxin substrates indicated that RAW264 cells have only one major pertussis toxin substrate identified as G_i-2 . A similar protein was also identified in bovine brain. It is likely that G_i-2 is the guanine nucleotide binding protein that couples chemotactic receptors to an effector protein such as phospholipase C or ion channels. A second family of guanine nucleotide binding proteins that are not substrates for either cholera toxin or pertussis toxin has been identified in RAW264 cells. These membrane proteins were identified by radiolabelling the proteins with 3H -methyl-AdoMet in the presence of GTP. The properties of these proteins and the details of their identification are presented in more detail in the report for project #Z01 MH 00931-14 LGCB. The RAW264 system will be used to test the role these proteins may have in chemotaxis.

Significance of Biological Research to the Program of the Institute:

Several reports have shown that stress-induced neuropeptides modulate immunological activities and that leukocytes have receptors for beta-endorphin and other neuropeptides. Chemotaxis is an important component of the immunological response, and it has been shown that human monocytes exhibit chemotaxis to met-enkephalin and beta-endorphin. Injection of beta-endorphin into the rat cerebral ventricle results in the immigration of macrophage-like cells, indicating that chemotaxis to beta-endorphin can occur in vivo. Identification of the steps involved in chemotaxis would provide a basis for

the development of strategies to counteract stress-induced immunological dysfunction.

Guanine nucleotide binding proteins are important components of signal transduction by both hormones and chemoattractants. Brain is also one of the richest sources of guanine nucleotide binding proteins, suggesting that these proteins may be important regulators of brain function. Studies of interactions between receptors, guanine nucleotide binding proteins, and various effector systems, such as adenylate cyclase, should improve our understanding of signal transduction mechanisms in cells.

Mammalian cell chemotaxis is also important in the development of the nervous system, inflammation and wound healing, and chemotaxis is a behavioral response at the cellular level. Studies of bacterial chemotaxis from the laboratories of Koshland and Adler have shown that bacteria have "memory" and adapt to their environment, and progress has been made in explaining these concepts in molecular terms. The mammalian cell line model for chemotaxis that we have developed provides a mammalian system to test concepts developed from bacterial chemotaxis and to study the biochemical reactions involved in signal transduction.

Proposed Course of Research:

Future work will be directed toward the identification of biochemical components of chemotaxis. These problems will be approached by a combination of biochemical and molecular biology techniques.

Publications:

Backlund PS Jr, Aksamit RR, Unson CG, Goldsmith P, Spiegel AM, Milligan G. Immunochemical and electrophoretic characterization of the major pertussis toxin substrate of the RAW264 macrophage cell line, *Biochemistry*, 1988;27:2040-6. 1988.

Goldsmith P, Backlund PS Jr, Rossiter K, Carter A, Milligan G, Unson CG, Spiegel A. Purification of heterotrimeric GTP-binding proteins from brain: identification of a novel form of G_0 , *Biochemistry* 1988; in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00943-07 LGCB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Pathways of Methionine and Threonine Metabolism and Their Control in Higher Plants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.	J. Giovannelli	Research Chemist	LGCB	NIMH
	S.H. Mudd	Chief, Section on Alkaloid Biosynthesis	LGCB	NIMH
	A.H. Datko	Biologist	LGCB	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Alkaloid Biosynthesis

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

1.2

PROFESSIONAL

1.2

OTHER

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Z01 MH 00943-07 LGCB has been terminated because the Section on Alkaloid Biosynthesis has been closed.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02321-03 LGCB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

DNA Methylation and Gene E

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. G.L. Cantoni Chief, Laboratory of General and Comparative Biochemistry LGCB NIMH

A. Razin Visiting Scientist, The Hebrew University
Jerusalem, Israel

Others: S. Agostini Guest Researcher LGCB NIMH
T. Gomi Visiting Fellow LGCB NIMH

COOPERATING UNITS (if any)

Department of Cellular Biochemistry, The Hebrew University, Hadassah Medical School, Jerusalem, Israel; Department of Human Biopathology, University of Rome, La Sapienza, Rome, Italy

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have shown earlier that the transient DNA hypomethylation which is observed when murine erythroleukemia cells (MELC) are induced to differentiate is due to a novel enzymatic mechanism which in the absence of DNA replication, brings about a modification in the pattern of DNA methylation by the specific removal of methyl cytidine (mC) residues and their replacements by cytidine. We have now found that treatment of MEL cells with 3-DZA (3-deaza-Ado) and homocysteine during the first 20 hours after induction with HMBA will completely inhibit the expression of the differentiated state (measured at 72-96 hours). If treatment with 3-deaza-Ado was delayed until 24 hours after induction differentiation was not affected. We have also established that the inhibition of differentiation produced by 3-DZA + Hcy is accompanied by the inhibition of hypomethylation induced by HMBA. Moreover, the inhibition of differentiation caused by 3-DZA + Hcy is strictly correlated with the length of the exposure to these compounds: if 3-DZA + Hcy are added together with the inducer but removed 8 hrs later there is no effect either on differentiation or on DNA hypomethylation; if the cells are exposed for 12 or 18 hrs after induction differentiation is inhibited by 20 and 90%, respectively, and the hypomethylation is inhibited correspondingly. Exposure to 3-DZA + Hcy for the first 20 hrs after induction with HMBA results in complete inhibition of differentiation and the loss of methylcytidine is entirely prevented. We have also shown that if addition of 3-DZA + Hcy is delayed with respect to the addition of HMBA its effects are progressively diminished. The striking correspondence in the timing of the inhibition of differentiation and of the HMBA induced DNA hypomethylation produced by 3-DZA + Hcy adds weight to the hypothesis that this limited and specific modification of DNA structure is necessary but probably not sufficient for the expression of the differentiated genotype.

Other Investigators:

A. Levine	The Hebrew University, Jerusalem, Israel
T. Kafri	The Hebrew University, Jerusalem, Israel

Project Description:

Murine erythroleukemia (MEL) cells can be induced to differentiate in vitro by a variety of agents. It has become increasingly clear that the decision to "switch" from a state of continuous proliferation to terminal differentiation upon chemical induction involves multiple events, such as changes in membrane permeability, chromatin structure, DNA methylation and expression of oncogens. Clearly these changes reflect a major alteration in the repertoire of genes that are expressed in MEL cells after induction.

Earlier studies by Marks and his colleagues have established that terminal differentiation, measured by the synthesis of hemoglobin, is a relatively late event that takes place 72 to 96 hrs after induction. Terminal differentiation is preceded by "commitment" an irreversible change that conditions the cells to differentiate even if the inducer is removed. Oishi and his collaborators have in fact isolated and characterized two different cytoplasmic factors that act synergistically in the process of MEL cell differentiation.

Cellular differentiation involves both activation and silencing of genes a process that has been shown in many instances to be associated with a change in the pattern of DNA methylation.

It is self-evident that a change in the pattern of DNA methylation involves at least two steps, namely loss of methylated cytidines at some sites of the genome and methylation of previously unmethylated cytidines at other sites.

We have previously shown that when MEL cells are exposed to a variety of chemical agents capable of inducing terminal differentiation their DNA undergoes a transient genome-wide hypomethylation. Earlier work from this laboratory led to the definition of a novel mechanism responsible for the transient and quickly reversible hypomethylation that accompanies induction of terminal differentiation in MEL cells. We have shown conclusively that transient DNA demethylation is due to a unique mechanism whereby a substantial fraction of 5-methylcytosine residues are specifically replaced by cytosine. The ratio of 5-methylcytosine: cytosine in DNA extracted from cells 12 hours after induction is strikingly lower than that from untreated cells or from cells induced for 24 hrs. The timing of this transient hypomethylation indicates that the phenomenon occurs in the absence of DNA replication. The removal of 5-methylcytosine and its replacement by cytosine is specific both with regard to the base and its position in the sequence of deoxynucleosides in DNA. This conclusion is based on the demonstration that the cytosine residues that become incorporated into DNA in replacement of 5-methylcytosines were incorporated specifically into methylatable (or CpG) sites.

In biochemical terms this discovery indicates that the modulation of the pattern of DNA methylation during differentiation in MEL cells involves at least

two enzymatic steps catalyzed respectively by methylcytidine replacase and a de novo DNA methylase.

The long term objective of this project is to identify the enzymatic activities responsible for the changes in the pattern of DNA methylation as related to cellular differentiation.

3-Deazaadenosine (3-deaza-Ado) has been extensively studied in this lab for its ability to function as a substrate of AdoHcyase and give rise in vivo to a congener of adenosylhomocysteine endowed with specific biochemical characteristics. In order to examine the relationship between biological methylation and the novel 5-methylcytosine replacement reaction the effect of the administration of 3-deaza-Ado on MEL cell differentiation was examined.

It was found that treatment of MEL cells with 3-deaza-Ado and homocysteine during the first 20 hours after induction with HMBA or DMSO will completely inhibit the expression of the differentiated state (measured at 72-96 hours). By contrast when treatment with 3-deaza-Ado was delayed until 24 hours after induction differentiation was not affected. The effect of 3-deaza-AdoDZA was specific (neither adenosine nor deazaaristeromycin had any effect) and required the presence of homocysteine, a result that indicates conclusively that the effect is mediated by adenosylhomocysteinase. We have also established that the inhibition of differentiation is accompanied by the inhibition of hypomethylation induced by HMBA. Moreover, the inhibition of differentiation caused by 3-DZA + Hcy is strictly correlated with the length of the exposure to these compounds: if 3-DZA + Hcy are added together with the inducer but removed 8 hrs later there is no effect either on differentiation or on DNA hypomethylation; if the cells are exposed for 12 or 18 hrs after induction, differentiation is inhibited by 20 and 90%, respectively, and the hypomethylation is inhibited correspondingly. After exposure to 3-DZA + Hcy for 20 hrs the loss of methylcytidine is entirely prevented. We have also shown that if addition of 3-DZA + Hcy is delayed with respect to the addition of HMBA its effects are progressively diminished.

This transient decrease in 5-methylcytidine that is induced by HMBA is not affected by exposure of the cells to protein synthesis inhibitors such as cycloheximide, whereas differentiation is substantially inhibited under these conditions. The striking correspondence in the timing of the inhibition of differentiation and of the HMBA induced DNA hypomethylation produced by 3-DZA + Hcy adds weight to the hypothesis that this limited and specific modification of DNA structure is necessary but probably not sufficient for the expression of the differentiated genotype.

Significance to the Program of the Institute:

Biological methylation underlies many different physiological events. A role for biological methylation has been proposed for such diverse phenomena as memory and chemotaxis in bacteria, repair of cellular damage due to aging, fruit ripening, membrane and receptor function, synthesis and maturation of messenger RNA and gene expression.

In addition, there is a growing body of experimental evidence that suggests that alteration in specific methylation reactions may be important in disease. The results from well controlled clinical studies have documented the efficacy of S-adenosylmethionine in depression, in chronic arthritis and in liver diseases. The biochemical basis for

these effects is entirely unknown and the search for suitable explanations for these pharmacological effects presents a formidable challenge.

All methylation reactions utilize S-adenosylmethionine as the methyl donor and are inhibited by S-adenosylhomocysteine. The great number and variety of reactions involving these intermediates has provided both an opportunity and an obstacle to the elucidation of the controlling role of methylation reactions in the different phenomena listed above. Continued efforts directed to unraveling the physiological significance and control of methylation reactions is fundamental to progress in this important area of research.

Publication:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 MH 01037-20 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of the Cell Membrane in Cellular Organization: A Molecular Study

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. M. Neville, Jr.	Chief, Sec. on Biophy. Chem.	LMB, NIMH
Others:	T. H. Hudson	Staff Fellow	LMB, NIMH
	J. W. Marsh	Staff Fellow	LMB, NIMH
	K. Srinivasachar	Visiting Associate	LMB, NIMH
	K.-H. Jung	Visiting Fellow	LMB, NIMH
	Yan Bai	Guest Researcher	LMB, NIMH
	Bei-Fen Shen	Guest Researcher	LMB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Biophysical Chemistry

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

7

PROFESSIONAL:

5

OTHER:

2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The general aim of this project is to determine the chemical interactions and energetics such as membrane potential and pH and ion gradients which are involved in the insertion of proteins into cellular membranes and/or the translocation of proteins across cellular membranes. The events are studied from the initial receptor binding to the final physiologic response or pathological response in the case of toxins such as ricin, colicins, diphtheria and tetanus toxins. Utilizing basic data from such studies immunotoxins (toxins linked to monoclonal antibodies) are constructed to serve as a new class of pharmacologic reagents to eliminate unwanted cell types such as cancer cells or T-4 lymphocytes in AIDS infections, or to manipulate specific cells such as T cell subsets to correct imbalances which exist in autoimmune diseases which can affect the CNS such as multiple sclerosis and lupus and cause psychosis. In addition immunotoxins continue to prove useful in deminishing the incidence of graft-versus-host-disease following bone marrow transplantation and thus will also have utility in enzyme replacement therapy and organ transplantation.

Project Descriptions:

The general aim of this project is to determine the chemical interactions and energetics such as membrane potential and pH and ion gradients which are involved in the insertion of proteins into cellular membranes and/or the translocation of proteins across cellular membranes. The events are studied from the initial receptor binding to the final physiologic response or pathological response in the case of toxins such as ricin, colicins, diphtheria and tetanus toxins. Utilizing basic data from such studies immunotoxins (toxins linked to monoclonal antibodies) are constructed to serve as a new class of pharmacologic reagents to eliminate unwanted cell types such as cancer cells or T-4 lymphocytes and macrophages in AIDS infections, or to manipulate specific cells such as T cell subsets to correct imbalances which exist in autoimmune diseases which can affect the CNS such as multiple sclerosis and lupus and cause psychosis. In addition immunotoxins continue to prove useful in diminishing the incidence of graft-versus-host-disease following bone marrow transplantation and thus will also have utility in enzyme replacement therapy and organ transplantation.

Major Findings:

A new class of protein or protein-macromolecule crosslinking reagents have been synthesized which are cleavable under mild acidic conditions. The hydrolytic rates are such that these crosslinkers are cleaved within minutes or hours at the pH of acidified cellular vesicles, pH 5.4, yet are 100 times more stable at the intravasacular pH of 7.4, and 1000 times more stable at a storage pH of 8.4. The crosslinkers are based on acetal, ketal and orthoester groups which undergo hydrolysis by specific acid catalysis yet, are resistant to base or nucleophile catalyzed hydrolysis. Coupling functionalities include maleimides and N-hydroxy succinimide esters.

Diphtheria toxin based immunotoxins are 50 fold more potent when constructed with an acid cleavable crosslinker DMEP. This immunotoxin at pH 5.5 dissociates into DT and antibody with a half-life of 42 minutes. The dissociation within the acidified cellular endosome compartment apparently removes steric constraints and enhances antibody mediated toxicity. The toxin binding domain of immunotoxins have also been reversibly blocked utilizing DMEP.

The mouse's natural resistance to diphtheria toxin has been overcome for target cells by coupling diphtheria toxin to an antibody. These immunotoxins are effective *in vivo*. Utilizing shifts in temperature, pH and ammonium ion it has been demonstrated that antibody mediated routing in murine cells is different than DT receptor mediated routing in sensitive cells.

Significance to Biomedical Research and the Program of the Institute:

The reversible blockade of toxin binding sites with cleavable crosslinkers allows for the construction of immunotoxins for *in vivo* use. Toxin binding sites are blocked in the extracellular compartment reducing non-target cells and systemic toxicity. However when unblocked intracellularly these sites enhance efficacy and widen the therapeutic margin.

The ability to extend immunotoxins to *in vivo* use has particular significances in eliminating the reservoir of infected cells (macrophages and T4⁺ lymphocytes) in AIDS. Manipulation of other immune system subsets may be of use in the therapy of autoimmune diseases such as lupus psychosis (Bonfa et al., 1987).

Knowledge of alternate routing schemes for toxins and immunotoxins is providing knowledge on why toxin binding domain deletions reduce immunotoxin efficacy.

Proposed Course:

Animal studies on *in vivo* use of immunotoxins constructed with cleavable crosslinkers will be undertaken. Further studies on the role of toxin binding domain and routing through intracellular compartments are planned for a series of toxins and immunotoxins. The ability of diphtheria toxin based immunotoxins to modify *in vivo* immunologic function in a murine model system will continue to be explored.

Publications:

Filipovich AH, Vallera A, Youle RJ, Haake R, Blazar BR, Arthur D, Neville DM, Jr, Ramsay NK, McGlave P, Kersey JH. Graft-versus-host disease prevention in allogeneic bone marrow transplantation from histocompatible siblings. A pilot study using immunotoxins for T cell depletion of donor bone marrow, Transplantation 1987;44: 62-9.

Hudson TH, Neville DM, Jr. Temporal separation of protein toxin translocation from processing events, J Biol Chem 1987; 262:16484-94.

Wellhöner HH, Neville DM, Jr. Tetanus toxin binds with high affinity to neuroblastoma x glioma hybrid cells NG 108-15 and impairs their stimulated acetylcholine release, J Biol Chem 1987; 262:17374-8.

Neville DM, Jr. Immunotoxins for *in vivo* therapy: Where are we? Ann NY Acad Sci 1987;507:155-64.

Marsh JW, Neville DM, Jr. Development of an immunotoxin with *in-vivo* efficacy for murine systems, Ann NY Acad Sci 1987; 507:165-71.

Hudson TH, Scharff J, Kimak MAG, Neville DM, Jr. Energy requirements for diphtheria toxin translocation are coupled to the maintenance of a plasma membrane potential and a proton gradient, J Biol Chem 1988; 263:4773-81.

Marsh JW, Neville DM Jr. A flexible peptide spacer increases the efficacy of holoricin anti-T cell immunotoxins, J Immunol 1988;140:3674-8.

Marsh JW, Srinivasachar K, Neville DM, Jr. Antibody-toxin conjugation. In: Frankel A, ed. Immunotoxins. Boston: Martinus Nijhoff . 1988;213-8.

Neville DM, Jr, Marsh JW. Methods for quantifying immunotoxin efficacy. In: Frankel A, ed. Immunotoxins. Boston: Martinus Nijhoff Publ. 1988;393-04.

Hudson TH, Neville DM, Jr. Enhancement of immunotoxin action: Manipulation of the cellular routing of proteins. In: Frankel A, ed. Immunotoxins. Boston: Martinus Nijhoff Publ. 1988;371-92.

Marsh JW. Antibody mediated routing of diphtheria toxin in murine cells results in a highly efficacious immunotoxin, J Biol Chem (in press).

Neville DM Jr, Srinivasachar K. Protein crosslinking reagents cleavable within acidified intracellular vesicles, US Patent (pending).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01035-20 LMB																	
PERIOD COVERED October 1, 1987 to September 30, 1988																			
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Process of Lysogeny																			
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI:</td> <td style="width: 30%;">H. A. Nash</td> <td style="width: 30%;">Chief, Sec. on Molecular Genetics</td> <td style="width: 10%;">LMB, NIMH</td> </tr> <tr> <td rowspan="4">Others:</td> <td>P. Kitts</td> <td>Visiting Associate</td> <td>LMB, NIMH</td> </tr> <tr> <td>M. Bruist</td> <td>Research Associate</td> <td>LMB, NIMH</td> </tr> <tr> <td>C.-C. Yang</td> <td>Visiting Fellow</td> <td>LMB, NIMH</td> </tr> <tr> <td>S. Goodman</td> <td>Staff Fellow</td> <td>LMB, NIMH</td> </tr> </table>			PI:	H. A. Nash	Chief, Sec. on Molecular Genetics	LMB, NIMH	Others:	P. Kitts	Visiting Associate	LMB, NIMH	M. Bruist	Research Associate	LMB, NIMH	C.-C. Yang	Visiting Fellow	LMB, NIMH	S. Goodman	Staff Fellow	LMB, NIMH
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	C.-C. Yang	Visiting Fellow	LMB, NIMH																
	S. Goodman	Staff Fellow	LMB, NIMH																
COOPERATING UNITS (if any) Laboratory of Molecular Genetics, NICHD; Department of Microbiology, University of Illinois, Urbana, IL																			
LAB/BRANCH Laboratory of Molecular Biology																			
SECTION Section on Molecular Genetics																			
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892																			
TOTAL MAN-YEARS: <div style="text-align: center; font-weight: bold;">4.85</div>	PROFESSIONAL: <div style="text-align: center; font-weight: bold;">3.85</div>	OTHER: <div style="text-align: center; font-weight: bold;">1.0</div>																	
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>																			
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p> We have discovered several new features of the mechanism of the <u>site-specific recombination</u> that integrates the DNA of the <u>bacterial virus lambda</u> into the chromosome of its <i>E. coli</i> host. We showed that the two strand-exchanges that comprise the crossover not only are separated in time and space but have a fixed order. We have determined that this polarity is dictated not by local DNA sequence preferences but by the global structure of the synaptic complex. We have initiated a <u>kinetic study</u> of recombination by determining the dependence of the rate of the reaction upon the concentration of one of the DNA species. The results indicate that recombination is a surprisingly low affinity reaction. An accessory factor is needed for efficient recombination; we have shown that this protein causes <u>bending of DNA</u> upon binding. </p>																			

Objectives:

There are only four physiologically significant programmed transformations of DNA: replication of DNA to permit growth by division, transcription of DNA into RNA to permit gene expression, repair of accidental alterations of DNA to preserve the fidelity of the genome, and rearrangement of DNA to permit expansion of the information content of the genome. This project is concerned with understanding the mechanism of the last of these transformations, genetic recombination. We want to know how a cell manages to align two DNA sequences and to arrange the reciprocal transfer of genetic information from one to another. We have taken a biochemical approach to this problem and have studied one particular genome rearrangement - the integration of DNA of the bacterial virus lambda into the chromosome of its host, *E. coli*. In the past we have made substantial progress in determining the overall features of this reaction and the biochemical principles that underlie some of its individual steps. Our current work seeks to answer questions about the order in which DNA strands are exchanged, the rates for various steps in the pathway, and the way in which an accessory protein assists the recombinase in initiating the reaction.

Major Findings:

Our earlier work had shown that the breakage and rejoining of DNA during lambda integrative recombination takes place in two steps. First, one strand from each parent is broken, exchanged, and rejoined; this creates an intermediate called a Holliday structure (named for the scientist who first postulated this intermediate). Subsequently, the Holliday structure is resolved into a completed recombinant by breakage, exchange, and rejoining of a second strand from each parent. We have now determined that there is a fixed order to the two sets of strand exchanges. Since the DNA recombination loci (attachment sites) contain asymmetric sequences, one can assign a polarity to each locus; we find that the exchange occurs on the left before it occurs on the right. We have shown this in two ways. First, the consequence of blocking strand exchange by substituting phosphorothioate for phosphate in the core region of each attachment site (the place where strand exchanges occur) is much more severe when the block is placed on the left than when it is placed on the right. Second, Holliday structures isolated during unblocked recombination reactions have all been made by left-sided exchange. We have determined the features of the attachment site that govern this asymmetry. Our data rule out the participation of local DNA sequences within the core and show that the asymmetry is fostered by protein binding sites that lie 50 to 150 base pairs away from the core in the so-called arms of the attachment site. In our most decisive experiment, we have switched the position of these arms with respect to the core and found that the bias in strand exchange is reversed. This artifact not only proves that the bias in strand exchange reflects global rather than local features of the attachment site, it permits us to test another aspect of the recombination mechanism. Our earlier work had shown that identity between the sequences of two recombining attachment sites was more crucial at the left of the core than at the right. We proposed that this asymmetry comes about because Holliday structures are formed on the left and need to move after formation, a feat which is expected only when DNA sequences are identical. Our finding that Holliday structures normally form on the left supports this proposal but does not rule out a fortuitous relationship between these two features. Using the artificial construct that generates Holliday structures on the right, we have now retested the effect of positioning non-identities in the DNA sequence. We find that the critical region now lies at the right, proving that the position of Holliday structure formation dictates the location of essential homology.

Kinetic studies can reveal what step(s) limit the rate of a biochemical reaction. We have initiated a kinetic study by determining the dependence of the rate of *in vitro* recombination on the concentration of one attachment site. For this study we focused on *attB*, the attachment site of the *E. coli* host, because our earlier work showed that it functioned as a passive partner in the reaction, being captured by a nucleoprotein array assembled at the viral attachment site, *attP*. We

find that the rate of recombination is proportional to *attB* concentration over a wide range, including values as high as 500 nM. This implies that *attP* is not saturated with *attB* even though *attP* concentration is fixed in these experiments at only 6 nM. The failure to saturate could come about in two ways; when *attP* and *attB* come together to make a synaptic complex, either recombinant products arise at a very rapid rate or synaptic complexes have a great propensity to disassemble before strand exchange can occur. To distinguish these possibilities, we have examined the kinetics of recombination under conditions where the rate of conversion of synaptic complexes to products is artificially reduced. Such reduction is accomplished by using a pair of attachment sites that have non-identical core sequences, a strategy that reduces the rate of recombination by about three orders of magnitude. Under these conditions, *attB* still fails to saturate *attP*. We conclude that integrative recombination is a low affinity reaction in which the partner sites have, at best, a poor tendency to interact with one another.

Integrative recombination is carried out by two proteins - a virally encoded recombinase, Int, and an accessory protein encoded by the host, IHF. IHF is a sequence-specific DNA binding protein that this laboratory discovered because of its role in recombination. Genetic and biochemical studies have made it clear that IHF is a ubiquitous accessory protein in *E. coli*, playing important roles in processes as diverse as the packaging of viral DNA, the initiation of replication of plasmids, the transposition of diverse genetic elements, and the control of gene expression. Our previous studies of the role of IHF in recombination suggested that this protein helped to arrange *attP* into an ordered nucleoprotein array. One way in which IHF might do this would be to bend DNA so as to expedite the folding of *attP* into a compact form in which Int protomers could interact with one another. We have now tested the capacity of IHF to bend DNA. The test was based on the electrophoretic mobility of a set of fragments of identical length and sequence, each of which is permitted so as to carry an IHF binding site at a different distance from the ends of the fragment. The migration of IHF complexes with this set of fragments is expected to vary only if IHF bends the DNA which it binds. To simplify the generation of the desired permuted set of fragments, we first constructed a novel vector that should prove generally useful for this kind of study. We then cloned an IHF binding site (extracted from *attP*) into this vector and determined the electrophoretic mobility of permuted fragments in the presence and absence of IHF. Our results show that the naked DNA is straight but that IHF introduces an easily detectable bend. We studied a second IHF site with this vector and came to the same conclusion. In each case, the position of the bend, determined from the member of the permuted set that exhibits the slowest mobility, mapped to the IHF site.

Significance to Biomedical Research and the Program of the Institute:

A current theme of great interest in molecular biology is the structure and function of multi-protein: multi-site complexes. These arrays are recognized as critical elements in all the important biological transformations of DNA, especially in animal cells where genome complexity is high. Although it is a prokaryotic reaction, lambda site-specific recombination epitomizes this kind of multi-component system. Our growing understanding of this system serves as a model for those systems in which the DNA anatomy and protein components are not yet elucidated. In particular, our investigation of the role of bending of DNA by sequence-specific binding proteins in the construction of nucleoprotein arrays serves as a paradigm on both technical as well as conceptual grounds.

Our studies of strand exchange establish several important features of the recombination reaction. Most importantly, we find that distant DNA sequences dictate the order of exchange of strands. Since these distant sites are needed for synapsis between recombining partners, we conclude that synapsis not only juxtaposes partners but holds them in a specific configuration that prompts one set of breakage and reactions before the other. All future investigations of synaptic complexes will need to incorporate our specific results.

The kinetic analysis of site-specific recombination is the first that has been attempted. Our initial results shows that this kind of analysis can yield answers which are both reliable and provocative. Especially challenging is our conclusion that recombining partners sense each other with only low affinity.

Proposed Course:

The project on the bias in strand exchange has been completed and prepared for publication. The study of the kinetics of recombination will be extended in several directions. We want to know whether the affinity which we measure with purified components applies to intracellular conditions. In addition we wish to compare other modes of site-specific recombination, e.g., excisive recombination, to see if low affinity is a universal trait. Finally, we plan to extend the concentration range so as to extract kinetic constants from the analysis and then study individual components of the reaction for their effect on these constants. The study of DNA bending by IHF protein has been completed and written up. We plan to challenge the basic conclusion of this study by attempting to substitute other proteins that bend DNA for IHF. In addition, we will try to characterize the way in which IHF binds to DNA so that we can understand and control its capacity to bend DNA.

Publications:

Nash HA, Robertson CA, Flamm E, Weisberg RA, Miller HI. Overproduction of *Escherichia Coli* integration host factor, a protein with nonidentical subunits, J Bact 1987; 169:4124-7.

Kitts PA, Nash HA. Homology-dependent interactions in phage λ site-specific recombination, Nature 1987;329:346-8.

Richet E, Abcarian P, Nash HA. Synapsis of attachment sites during lambda integrative recombination involves capture of a naked DNA by a protein-DNA complex, Cell 1988;52:9-17.

Robertson CA, Nash HA. Bending of the bacteriophage λ attachment site by *Escherichia coli* integration host factor, J Biol Chem 1988; 263: 3554-7.

Kitts PA, Nash HA. An intermediate in the phage lambda site-specific recombination reaction is revealed by phosphorothioate substitution in DNA, Nucleic Acids Res (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 02228-04 LMB
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Genetic Neurobiology of Drosophila		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: H. A. Nash Chief, Sec. on Molecular Genetics LMB, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Molecular Biology		
SECTION Section on Molecular Genetics		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: <div style="text-align: center; font-weight: bold;">1.15</div>	PROFESSIONAL: <div style="text-align: center; font-weight: bold;">0.15</div>	OTHER: <div style="text-align: center; font-weight: bold;">1.0</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="margin-top: 20px;"> <p>We have isolated mutants of <u>Drosophila melanogaster</u> that show an altered sensitivity to <u>general anesthetics</u> holathane. The sensitive mutants are very unstable but the resistant lines breed true. We are developing methods for the <u>genetic mapping</u> of these mutants and have made some preliminary observations on their pharmacology.</p> </div>		

Objectives:

We are interested in using mutants of *Drosophila melanogaster* to identify genes that encode or control components of the nervous system that are difficult to study with traditional pharmacological or biochemical approaches. There is reason to believe that the phenomenon of general anesthesia involves one or more such components. Although the response to general anesthetics can be rather specific - loss of pain sensation and consciousness but retention of autonomic function and simple reflexes - it has been hard to localize the action of anesthetics at either the anatomic, cellular, or molecular level. We are in the process of isolating and characterizing mutants with altered response to a general anesthetic in the expectation that some of these mutants will help highlight the target(s) of anesthetic action.

Major Findings:

We have previously established a screening procedure for determining in a semi-quantitative way the response of populations of fruit flies to the general anesthetic halothane. We have applied this procedure in order to select individuals with an abnormal response from amongst a population of mutagenized flies. While most candidate flies with unusual responses proved to be false positives, we have succeeded in identifying four resistant and two sensitive lines from over 20,000 mutagenized offspring. As expected from the experimental design of the mutagenesis, all the mutants appeared to be sex-linked. Initial characterization showed that these lines differed in their response to halothane but were distinguished from each other in their response to a second anesthetic, enflurane.

Our current efforts have been focused on characterizing these mutants first from a genetic and then from a pharmacological point of view. Unfortunately, the mutants have been difficult to work with and progress has been slow. The sensitive lines have been particularly troublesome; both lines rapidly acquired genetic modifiers that reduced the anesthesia response phenotype to barely detectable levels. Backcrossing to introduce unmutagenized autosomes to these lines has failed to recover the phenotype; the modifiers thus may be located on the X chromosome. Backcrosses which permit exchanges with an unmutagenized sex chromosome are underway. The resistant lines have proven more stable, although two of them show low viability. To map these mutations, we have had to work out simpler screening procedures for the anesthetic response that do not depend upon large numbers of flies. We have recently succeeded in developing such a test. In it, groups of ten flies are exposed to a standard dose of anesthesia, placed on a surface and allowed a fixed period of time to escape; the fraction of flies that remain on the surface measures their overall motor performance. This assay reproducibly distinguishes mutant from control stocks of flies and thus opens the way to assess the linkage of the anesthetic response mutation to easily scored genetic markers whose chromosomal location are known.

Significance to Biomedical Research and Program of the Institute:

The phenomenon of anesthesia offers insights into the neural mechanisms that underlie consciousness. As such, investigations into the mechanisms of anesthesia, i.e. the anatomic, cellular, and biochemical structures that are affected by anesthetic gases is germane to understanding higher order nervous processes. Our genetic studies represent a new approach to delving into anesthetic mechanisms. Of particular interest is our preliminary results that indicate that several mutants have altered responses to one anesthetic but not to another. This finding challenges the widely held belief that all general anesthetics work by the same mechanism and are simply distinguished by potency. It will be of interest to determine if this nonuniform response reflects a basic distinction in the actions of individual anesthetics; detailed genetic studies should provide an answer to this question.

Proposed Course:

We will continue to backcross our sensitive mutants in attempt to remove genetic modifiers from these lines. We will also pursue the genetic mapping of the resistant lines. We will assess not only the linkage of anesthetic resistance to visible markers but to the low viability that characterizes two of our lines. The genetic mapping should lead to the construction of isogenic lines that will make an ideal substrate for subsequent detailed pharmacologic and biochemical testing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 00934-16 LMB																												
PERIOD COVERED October 1, 1987 to September 30, 1988																														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Biochemical Basis of Peptide Receptor Activity																														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 10%; vertical-align: top;">PI:</td> <td style="width: 30%;">W. A. Klee</td> <td style="width: 30%;">Chief, Sec. on Regulatory Proteins</td> <td style="width: 30%;">LMB, NIMH</td> </tr> <tr> <td style="vertical-align: top;">Others:</td> <td>D. L. Newton</td> <td>Staff Fellow</td> <td>LMB, NIMH</td> </tr> <tr> <td></td> <td>T. Osugi</td> <td>Visiting Fellow</td> <td>LMB, NIMH</td> </tr> <tr> <td></td> <td>R. C. Rice</td> <td>Research Chemist</td> <td>LC, NIADDK</td> </tr> <tr> <td></td> <td>A. E. Jacobson</td> <td>Research Chemist</td> <td>LC, NIADDK</td> </tr> <tr> <td></td> <td>M. Nirenberg</td> <td>Chief, Lab. Biochem. Genetics</td> <td>LBG, NIHLB</td> </tr> <tr> <td></td> <td>P. Hargrave</td> <td>Professor of Opthamology</td> <td>University of Florida</td> </tr> </table>			PI:	W. A. Klee	Chief, Sec. on Regulatory Proteins	LMB, NIMH	Others:	D. L. Newton	Staff Fellow	LMB, NIMH		T. Osugi	Visiting Fellow	LMB, NIMH		R. C. Rice	Research Chemist	LC, NIADDK		A. E. Jacobson	Research Chemist	LC, NIADDK		M. Nirenberg	Chief, Lab. Biochem. Genetics	LBG, NIHLB		P. Hargrave	Professor of Opthamology	University of Florida
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>As a result of screening 46 antibodies directed against defined regions of rhodopsin, we identified a <u>monoclonal antibody</u>, which also recognizes <u>opiate receptors</u> from NG108-15 <u>neuroblastoma x glioma hybrid cells</u>. A peptide corresponding to the epitope of the antibody inhibits precipitation of opiate receptors by the antibody. A peptide from the homologous region of the porcine brain <u>muscarinic receptor</u> not only blocks the antibody, but also activates a number of <u>G-proteins</u> both in membranes and in solutions of the purified proteins. Homologous peptide fragments of other receptors also activate the appropriate G-proteins. These experiments suggest that we have identified an important region of the the <u>signal transmitting domain</u> of G-protein coupled receptors. In related experiments, we have shown that the alkylating GTP analogue <u>fluorosulfonylbenzoylguanosine</u> (FSBG) can be used as a reagent to inactivate G-proteins in natural membranes without affecting receptor numbers or effector enzymes directly. Thus, FSBG treatment of NG108-15 membranes prevents <u>bradykinin</u> stimulation of <u>phospholipase C</u> activity without changing receptor numbers or the intrinsic or <u>calcium</u> activated enzyme activity. Bradykinin sensitivity can be restored to the treated membranes by addition of purified G-proteins.</p>																														

Project Description and Major Findings:

There are many types of receptors which receive chemical signals from the cellular environment and transmit this information to the interior. A large group of these cell surface proteins, including muscarinic, alpha2-adrenergic, opiate and many other peptide receptors share strong structural and functional similarities. These receptors, which interact with a number of hormones and neurotransmitters, are coupled to the activation or inhibition of intracellular enzymes by GTP-binding regulatory proteins (G-proteins). In this manner, information is transmitted to a cell from neighboring cells and from the fluid environment. Our goal is to dissect this system into its component parts, and study each of the proteins and other components of the system in isolation and as a reconstituted functional entities. In the past year, experiments have been performed which have resulted in identification of the receptor domain that activates G-proteins and the demonstration that peptides corresponding to this domain stimulate the activity of purified G-proteins. In related studies, we have also developed a general method to inactivate G-proteins in membranes so as to then be able to reconstitute receptor function by adding the appropriate, unmodified, G-protein.

We have concentrated our efforts on two of the G-protein-coupled receptors: opiate and bradykinin. The neuroblastoma x glioma hybrid cell line, NG108-15, is richly endowed with both of these receptors. They are known to function through different G-proteins and to ultimately affect different cellular processes. The cells express opiate receptors of a single type, namely δ . The δ receptors were shown to be coupled, as inhibitors, to adenylate cyclase both in these cells and in brain tissue. Activation of the receptors with opiates or opioid peptides reduces cellular cyclic AMP levels and thereby lowers the extent of phosphorylation of many cellular enzymes. In analogy to the addictive process, the cells become tolerant to and dependent upon opiates after prolonged exposure. This adaptive process is due to a gradual increase in adenylate cyclase activity which serves to maintain normal cyclic AMP levels in the continued presence of opiates. With opiates such as morphine, adaptation occurs in the absence of changes in receptor number. Other opioids, such as the enkephalins, produce receptor down-regulation as well as increased adenylate cyclase activity upon chronic exposure. Bradykinin receptors are known to activate phospholipase C so as to release inositol trisphosphate and raise free Ca ion levels. Thus, the two receptor prototypes are coupled to one or the other of two major second messenger systems: calcium ions and cyclic AMP.

We have over the past few years developed procedures for the solubilization of receptors from membranes by extraction with the zwitterionic detergent CHAPS, and have isolated affinity labeled opiate receptors in a homogeneous state. Such receptors, because of the presence of covalently linked opiates are useful structural studies. They have proven to be particularly good tools for antibody screening experiments as well.

All receptors share two essential properties: they bind ligands, and transmit the information of whether or not an activating ligand is bound. Many receptors send information to one of several GTP-binding regulatory proteins (G-proteins) all of which have very similar amino acid sequences. This family of G-proteins includes at least 4 types of Gi, Go, and transducin. Receptors coupled to these proteins include, among others, opiate, muscarinic and bradykinin receptors and the retinal (or light) receptor, rhodopsin. Activation of G-proteins by agonist occupancy of these receptors ultimately results in activation, or inhibition, of one of several enzymes including phospholipase C, cyclic GMP phosphodiesterase and adenylate cyclase. We reasoned that receptors of this class, which must all interact with very similar regulatory proteins, might share structural features responsible for these interactions. The availability of a battery of monoclonal antibodies directed against defined regions of rhodopsin (developed by Dr. Paul Hargrave and his collaborators at the University of Florida), allowed an experimental test of the hypothesis. For this test we used opiate receptors from NG108-15 neuroblastoma X glioma hybrid cells specifically substituted with the synthetic opiate [3H]-3-methylfentanylisothiocyanate (superFIT). We found that one of the 46 anti-

rhodopsin monoclonal antibodies tested also recognizes opiate receptors. The epitope against which this antibody is directed corresponds to the fourth cytoplasmic segment of the rhodopsin molecule immediately following the seventh (putative) transmembrane helix. A peptide corresponding to this region, rhodopsin 310-321, blocks interaction of the antibody with both rhodopsin and opiate receptors. An amidated peptide corresponding to the homologous region of the porcine brain muscarinic receptor, residues 422-431 (N10L) also blocks the antibody. Perhaps surprisingly, the peptide stimulates the GTPase activity of a number of purified G-proteins, but to somewhat different extents depending on the protein. The G proteins studied to date include Gi-1, Gi-2 and Go purified from bovine brain and transducin from bovine retina. As might be expected, the muscarinic receptor derived peptide is more effective as a stimulator of the brain G-proteins than it is of transducin. Conversely, a homologous peptide corresponding to the photon receptor, rhodopsin, is much more effective as an activator of the GTPase activity of transducin than of the brain G-proteins. These experiments suggest that N10L and the homologous peptides of other receptors correspond to an important part of the receptor domain directly responsible for information transmission and show that it can work in a completely defined system. Thus, many experiments can now be performed using only purified proteins and peptides that should greatly clarify the mechanism of receptor action. As more receptor sequences become available much will be learned using this approach about receptor specificity and selectivity. We have found, for example, that other peptides, which display sequence similarities with portions of the third cytoplasmic loop of the G-protein coupled receptors, can also activate some of the G-proteins. These experiments suggest that the conformational change responsible for receptor activation as a result of agonist occupancy may consist of the assembly of a structure consisting of these parts of the third and fourth cytoplasmic domains.

Among the important unsolved problems related to trans-membrane signalling is the question of which G-protein is actually coupled to which receptor in the cell. Although reconstitution experiments with purified components in lipid vesicles are possible, and have been done, the results are disappointing in that it is clear that almost any G-protein can be induced to couple with almost any receptor in these unphysiological circumstances. We reasoned that reconstitution experiments performed with the actual cell membranes, in as nearly native state as possible, have a much better chance of demonstrating the specificity of receptor-G-protein interactions which must exist in the cell. We had previously shown that such reconstitutions are readily effected if the G-proteins were first inactivated *in-vivo* with pertussis toxin. However, some G-proteins, particularly many coupled to phospholipase C activation, are not inactivated by pertussis toxin treatment. A good example is the G-protein which couples bradykinin receptors to phospholipase C in NG108-15 cells. We have therefore sought to develop a general method to inactivate any G-protein without harming the membranes in which they function. The alkylating GTP analogue fluorosulfonylbenzoylguanosine (FSBG) was chosen as a likely reagent. This compound is very similar to GTP in shape and polarity and would be expected to bind at the GTP site of G-proteins. It also contains an alkylating group capable of forming covalent bonds with appropriately positioned nucleophiles on the protein. Treatment of NG108-15 membranes with FSBG results in the loss of bradykinin stimulation of phosphatidylinositol breakdown, as anticipated. Basal activity is largely unaffected and the enzyme can still be fully activated by calcium ions. Thus, FSBG treatment has not affected phospholipase activity directly. Furthermore, the number of bradykinin receptors is unchanged although these are of low affinity, as they would normally be in the presence of GTP. Final proof that FSBG treatment has inactivated the G-proteins was achieved by the demonstration that addition of a mixture of G-proteins purified from bovine brain restores the ability of bradykinin to stimulate phosphatidylinositol breakdown in FSBG treated membranes. Thus, use of this reagent should allow a more physiologically relevant test of the specificity of bradykinin and other receptors for individual G-proteins. These are currently being prepared in large enough amounts to allow such testing.

Significance to Biomedical Research and the Program of the Institute:

A major problem in biology is understanding the mechanism of signal-response coupling across cell membranes. Cells communicate with one another and with their environment largely by means of chemical messengers which are sensed by cell surface receptors and thereby elicit other chemical changes within the cell. The opioid peptides, bradykinin, and related substances, are important transmitters of information in the nervous system. An understanding of how brain cells transmit and use such information is essential to the design of rational therapy for mental illness.

Proposed Course:

We plan to continue our efforts to understand the molecular basis of signal transduction, with particular emphasis on opiate and bradykinin receptors and the regulatory proteins with which they interact. In the next year we hope to be able to use our newly discovered insight into the nature of the signaling domain of receptors to learn more about how receptors activate G-proteins. The approach may also prove useful to elucidate those aspects of receptor structure which determine affinity for particular G-proteins. It does not seem unreasonable to hope that a completely synthetic receptor may be prepared that reproduces not only the signaling properties of hormone action but also the high affinity interaction with specific G-proteins as well.

Publications:

Tocque B, Pfeiffer A, Klee WA. Transfer of functional opiate receptors from membranes to recipient cells by polyethylene glycol-induced fusion, FEBS Lett 1987; 222:327-31.

Kim C-O, Jacobson AE, Mattson MV, Rice KC, Bykov V, Rothman RB, Streaty RA, Klee WA, George C, Long JB. Probes for narcotic receptor mediated phenomena. 15. (3S,4S)-(+)-trans-3-methylfentanyl isothiocyanate, a potent site-directed acylating agent for the delta opioid receptors, J Med Chem (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01031-20 LNC
PERIOD COVERED October 1, 1987 through September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Conversion of Phenylalanine to Tyrosine		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI	Seymour Kaufman Chief Michael Davis Senior Staff Fellow Hans-Ulrich Siegmund Visiting Fellow Masao Hirose Visiting Associate John Giovannelli Research Chemist	LNC NIMH LNC NIMH LNC NIMH LNC NIMH LNC NIMH
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neurochemistry		
SECTION		
INSTITUTE AND LOCATION ADAMHA, NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 4.2	PROFESSIONAL: 4.2	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>In order for <u>phenylalanine hydroxylase</u> to catalyze a fully uncoupled reaction, the enzyme must be highly activated and it must have a substrate analogue, such as <u>tyrosine</u>, occupying the <u>catalytic site</u>.</p> <p>Pure phenylalanine hydroxylase catalyzes the unusual reaction of hydroxylation of <u>p-methyl-phenylalanine</u> to <u>p-hydroxymethyl-phenylalanine</u>. The rate-limiting step is not the same as it is for the hydroxylation of phenylalanine.</p> <p>Phenylalanine hydroxylase in <u>hepatoma cells</u> is in a highly activated state.</p>		

Project Description

The objective of this research project is the detailed description of the enzyme system that catalyzes the conversion of phenylalanine to tyrosine. The specific goal is the analysis of the structure, mechanism of action, and modes of physiological regulation of the essential components in the hydroxylation system. These components include phenylalanine hydroxylase, dihydropteridine reductase and tetrahydrobiopterin (BH_4).

One of the reasons why the regulation of this system is of special interest to neurochemists is that it can serve as a paradigm for the dynamic interaction between metabolic events in peripheral organs and the brain. When this interaction goes awry, as it does in classical phenylketonuria, it can lead to severe mental retardation.

Major Findings

During the last year, we have investigated several aspects of the mechanism of action of hepatic phenylalanine hydroxylase, as well as one facet of its regulation.

With respect to mechanism, we have studied in greater detail a phenomenon that we had discovered some years ago, i.e., the "uncoupled" reaction catalyzed by phenylalanine hydroxylase. We had found that in the presence of a substrate analogue, such as tyrosine, and an activator, such as lysolecithin, phenylalanine hydroxylase catalyzes the oxygen-dependent oxidation of tetrahydrobiopterin (BH_4). Under these conditions there is no net hydroxylation of the amino acid, i.e., BH_4 oxidation is "uncoupled" from hydroxylation. We had proposed that the uncoupled reaction proceeds along the same pathway as the coupled reaction. In support of this proposal, the uncoupled reaction has provided valuable insights into mechanism. From studies of this reaction, e.g., we found the first evidence that oxygen at the reduction level of peroxide is formed during enzyme catalysis.

Recently, data were published claiming that tyrosine is not required for the uncoupled reactions, a finding that led these workers to challenge the idea that the uncoupled reaction is closely related to the normal reaction catalyzed by phenylalanine hydroxylase.

A detailed study of the uncoupled reaction confirmed in all respects our original finding that the reaction is dependent on the addition of tyrosine. Based on our data, we conclude that the challenge mounted by these other workers was probably based on one of several types of artifact that they were studying.

Given the usefulness of the fully uncoupled reaction as a tool for studying the mechanism of the hydroxylation reaction, we also felt that it was important to go beyond our previous evidence showing that there is no net hydroxylation of tyrosine and to investigate the possibility that tyrosine undergoes a transient hydroxylation. We studied this possibility by carrying out the reaction in the presence of $^{18}O_2$ and examining the isolated tyrosine for ^{18}O content. The tyrosine contained no detectable ^{18}O , a finding that

strongly supports the conclusion that there is not even a transient hydroxylation of tyrosine during the course of the uncoupled reaction.

Another aspect of the mechanism of phenylalanine hydroxylase that we studied involved a detailed study of the hydroxylation of p-methyl-phenylalanine. Before purified phenylalanine hydroxylase was available, it was reported by Guroff and coworkers that crude phenylalanine hydroxylase could catalyze a remarkable reaction--the hydroxylation of p-methyl-phenylalanine on the methyl group. Since this earlier study used crude enzyme preparations, it was important to determine whether this reaction was indeed catalyzed by phenylalanine hydroxylase and not by a contaminating enzyme. Furthermore, because hydroxylation of a methyl group is so different from the normal hydroxylation catalyzed by phenylalanine hydroxylase, we felt that it was essential that the source of the oxygen be determined.

Our studies showed that pure rat liver phenylalanine hydroxylase can catalyze the conversion of p-methyl-phenylalanine to p-hydroxymethyl-phenylalanine. We also showed, using $^{18}\text{O}_2$ and H_2^{18}O , that the oxygen in the newly synthesized hydroxymethyl group is derived exclusively from molecular oxygen and not from water. We have also synthesized p-methyl-phenylalanine labelled in the methyl group with deuterium and showed that conversion of the deuterated substrate to the hydroxymethyl compound involves a large isotope effect, proving that the rate-limiting step in the hydroxylation of p-methyl-phenylalanine is different from the limiting step in the hydroxylation of phenylalanine.

During the last year we have initiated studies of the properties and regulation of phenylalanine hydroxylase in hepatoma cells. Both the physical and catalytic properties of the enzyme in these cells indicates that the enzyme is in an unusually activated state compared to its relatively low-activity state in normal hepatocytes. Preliminary evidence is consistent with the hydroxylase in hepatoma cells being more highly phosphorylated.

Significance to Biomedical Research and Proposed Course of Project:

Our new results on the completely uncoupled reaction catalyzed by phenylalanine hydroxylase have consolidated our earlier evidence showing that an amino acid analogue, like tyrosine, is essential for this reaction to occur. Our present data show that under these conditions, the reaction is indeed uncoupled: not only is there no net hydroxylation of tyrosine but there is no evidence for even a transient hydroxylation. These results provide further insight into the roles of the regulatory and catalytic domains in the functioning of the hydroxylase. Tyrosine occupies the catalytic domain and yet it is not functioning as a substrate that can be hydroxylated. Rather, it must be functioning as a modulator of the activity. In other words, certain aspects of regulation are mediated at the catalytic domain!

The ability of phenylalanine hydroxylase to catalyze the conversion of p-methyl-phenylalanine to p-hydroxy-phenylalanine and the demonstration that molecular oxygen is the source of the oxygen in the product places phenylalanine hydroxylase in the group of enzymes that can hydroxylate a

saturated carbon atom. The significance of this finding is that all of the other enzymes in this group appear to use a ferryl ion species, FeO_2^+ , as the hydroxylating agent. Our data, therefore, indicate that this species may also be involved in the phenylalanine hydroxylase-catalyzed hydroxylation reactions. We plan to carry out additional experiments to determine whether a ferryl ion species is involved in the reaction.

The data showing that phenylalanine hydroxylase in hepatoma cells is in a highly activated state, suggestive of it being more fully phosphorylated, raises questions about whether this unusual state of activation is characteristic of phenylalanine hydroxylase in tumor cells. We plan to try to determine whether phenylalanine hydroxylase in hepatoma cells is actually more highly phosphorylated and if it is, whether this is due to a higher activity of protein kinases or a lower activity of protein phosphatases.

Publications:

1. Kaufman S. Phenylalanine 4-monooxygenase from rat liver. In: Kaufman S, ed. *Methods in Enzymology*. Academic Press, Orlando, Fl., 1987;3-17.
2. Kaufman S. The regulation of hepatic phenylalanine hydroxylase by phosphorylation. In: *The Roots of Modern Biochemistry*, Edited by Kleinkauf, H., VanDohren, U., Janenick, L. Lipman Memorial Symposium, Berlin, FRG, 1987.
3. Kaufman S. The enzymology of the aromatic amino acid hydroxylases. In: *Amino Acides in Health and Disease: New Prespectives*, UCLA Symposia on Molecular and Cellular Biology held at Keystone, Co., May 30-June 4, 1986 Edited by Kaufman, S. Alan R. Liss, Inc., New York, 1987;205-32.
4. Kaufman S. Aromatic amino acid hydroxylases. In: *The Enzymes* Edited by Boyer, P.D., Krebs, E.G. Academic Press, Orlando, Fl., Vol. 18, 1987;217-81.
5. Parniak MA, Davis MD, Kaufman S. Effect of alkaline pH on the activity of rat liver phenylalanine hydroxylase. *J. Biol. Chem.* 1988;263:1223-30.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01032-20 LNC

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biosynthesis of Catecholamines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	Seymour Kaufman	Chief	LNC NIMH
	Dominique Pigeon	Visiting Fellow	LNC NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

ADAMHA, NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.2

PROFESSIONAL:

1.2

OTHER:

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A new procedure has been developed for the purification of tyrosine hydroxylase from a pheochromocytoma cell culture. Some differences in enzymatic properties have been demonstrated between this enzyme and that from rat striatum.

Project Description:

The objective of this research project is the detailed description of the hydroxylation reactions that are involved in the biosynthesis of the neurotransmitters, dopamine and norepinephrine. Recently, we have been focusing on tyrosine hydroxylase from brain and pheochromocytoma in order to further clarify the molecular mechanism of activation of this enzyme by phosphorylation.

Major Findings:

Rat pheochromocytoma tyrosine hydroxylase (TH) has been purified to homogeneity from a large-scale culture of PC18 cells, a sub-clone of PC12. The TH level in the cells is already high but it has been further increased by induction with a glucocorticoid and an adrenalin agonist. The purification procedure was a modification of the one previously applied to the tumor enzyme.

A measurement of substrate specificity revealed that phenylalanine is a poorer substrate for TH purified from the pheochromocytoma cells than it is for TH in a striatum extract. This finding raises questions about differences in properties between the brain enzyme and the enzyme from this tumor. Attempts are in progress to purify TH directly from the corpus striatum.

These purified enzymes will also be used to further characterize the phosphatase activity that we have found in rat brain as well as in further studies of the regulation of this phosphatase by BH_4 and its precursor, GTP.

Significance to Biomedical Research and Proposed Course of Project:

The characterization of a phosphatase that catalyzes the dephosphorylation-mediated deactivation of tyrosine hydroxylase should enable us to obtain a much more complete picture of how the state of phosphorylation of tyrosine hydroxylase affects its activity. The demonstration that the phosphatase is inhibited by GTP and activated by BH_4 suggests a heretofore unsuspected way in which the coenzyme for the hydroxylase, BH_4 , can affect the activity of the hydroxylase. This observation has already suggested new interpretations for some puzzling old data in the literature.

We plan to continue to characterize this phosphatase and to study its interaction with tyrosine hydroxylase.

Publication:

1. Nelson TJ, Kaufman S. Activation of rat caudate tyrosine hydroxylase phosphatase by tetrahydropterins. J. Biol. Chem. 1987;262:16470-5.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01038-20 LNC

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Phenylketonuria and Other Diseases Caused by Defects in Biotpterin-Dependent Enzymes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	Seymour Kaufman	Chief	LNC NIMH
	Sheldon Milstien	Research Chemist	LNC NIMH
	Keiko Tanaka	Guest Worker	LNC NIMH

COOPERATING UNITS (if any)

Stanley Rapoport, Chief LN NIA

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

ADAMHA, NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.7

PROFESSIONAL:

1.7

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Further studies on BH_4 levels in the CSF of patients with Alzheimer's disease have uncovered a subgroup with movement disorders that have marked decreases.

A possible role of BH_4 in hematopoiesis has been discovered.

Project Description:

The goal of this research project is the detailed description, at the molecular level, of diseases caused by defects in components of the aromatic amino acid hydroxylating systems.

Major Findings

Preliminary short-term trials of BH_4 therapy in seven patients with Alzheimer's disease and low CSF BH_4 levels have been completed. Planning is now under way for a longer term trial to be carried out in a subgroup of these patients who have movement disorders and very low CSF BH_4 concentrations.

The human erythrocyte contains the enzymes for BH_4 biosynthesis. However, there are no known functions for BH_4 in the erythrocyte. Using mouse erythroleukemia cells as a model system, we have found that BH_4 synthesis is necessary for cell proliferation. Agents which cause the cells to differentiate and produce hemoglobin also turn off BH_4 synthesis. Specific inhibitors of BH_4 synthesis inhibit DNA synthesis but do not cause differentiation. The site of action of BH_4 in these cells remains to be elucidated. However, these findings suggest that the hematopoiesis status in BH_4 -deficient patients should be examined more closely.

Significance to Biomedical Research and Proposed Course of Project:

Work is under way to isolate cDNA clones to the enzymes of BH_4 biosynthesis to explore the molecular basis of alterations of BH_4 levels in variant forms of phenylketonuria as well as in Alzheimer's disease. Long term replacement therapy in cases of Alzheimer's disease should yield clues as to whether or not BH_4 deficiency is responsible for or a consequence of the disease and whether a deficiency of BH_4 -dependent neurotransmitters plays any role in dementia.

Publications:

1. Milstien S, Kaufman S. The oxidation of apomorphine and other catechol compounds by horseradish peroxidase. Relevance to the measurement of dihydropteridine reductase activity. Biochim. Biophys. Acta 1987;923: 333-8.
2. Irons M, Levy HL, O'Flynn ME, Stack CV, Langlais PJ, Butler IJ, Milstien S, Kaufman S. Folinic acid therapy in the treatment of dihydropteridine reductase deficiency. J. Pediat. 1987;110:61-7.
3. Kaufman S. Classical phenylketonuria and its variants caused by defects in bipterin metabolism. In: Kaufman S. ed. Amino Acids in Health and Disease: New Perspectives, UCLA Symposia on Molecular and Cellular Biology held in Keystone, CO, May 30-June 4, 1986. New York: Plenum Publishing Corp., 1987;517-38.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01039-20 LNC
PERIOD COVERED October 1, 1987 through September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pteridine Biosynthesis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI	Sheldon Milstien Seymour Kaufman	Research Chemist Chief LNC NIMH LNC NIMH
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neurochemistry		
SECTION		
INSTITUTE AND LOCATION ADAMHA, NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.6	PROFESSIONAL: 0.6	OTHER:
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither		
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>The terminal three enzymes in the pathway for <u>tetrahydrobiopterin</u> (BH₄) biosynthesis have been isolated and purified to homogeneity from rat brain. Antibodies have been prepared to each and studies are now underway using these antibodies to screen a DNA library in order to isolate DNA probes.</p>		

Project Description:

One important factor that regulates neuronal synthesis of the neurotransmitters, dopamine, norepinephrine, epinephrine, and serotonin is the availability of BH_4 , the coenzyme required in the rate limiting step of the synthesis of these neurotransmitters. In several pathological conditions, including Alzheimer's disease, Parkinsons disease, and dystonia, it has been reported that CNS BH_4 levels are decreased. A more complete understanding of the factors that regulate cellular levels of BH_4 is necessary to elucidate the possible role of alterations of BH_4 concentrations in the pathophysiology of these disorders as well as in normal neuronal function.

Major Findings:

The purification and preparation of specific antibodies to 6-pyruvoyl tetrahydropterin reductase have enabled us to clarify the role of this enzyme in BH_4 biosynthesis. In addition, this reductase was discovered to be similar to a class of non-specific keto reductases for which no previous physiological role had been discovered.

Significance to Biomedical Research and Proposed Course of Project:

Specific assays have been developed for the measurement of the activities of all of the enzymes of BH_4 biosynthesis in brain. Experiments are under way to determine the normal levels of these enzymes in human brain autopsy samples. We also plan to use the specific antibodies to further characterize the respective antigens by Western blotting techniques in brain samples from normals as well as Alzheimer patients.

Publications:

1. Milstien S., and Kaufman S. Regulation of biopterin biosynthesis in the rat. In: Blair JA, ed. Chemistry and Biology of Pteridines, Berlin, Walter de Gruyter and Co., 1987;753-757.
2. Milstien S. Tetrahydrobiopterin. Other physiological roles besides aromatic amino acid hydroxylation? In: HCh Curtius, NN Blau, RA Levine, eds., Unconjugated Pterins and Related Biogenic Amines Berlin, New York, W. de Gruyter, 1987;49-65.
3. Milstien S, Kaufman S. The regulation of biopterin biosynthesis in the rat. In: Blair JA, ed. Chemistry and Biology of Pteridines, Berlin-New York: Walter de Gruyter & Co, 1987;753-7.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01040-20 LNC
PERIOD COVERED October 1, 1987 through September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Biology of the Pterin-Dependent Hydroxylases and Ancillary Enzymes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI	Seymour Kaufman	Chief
	Sheldon Milstien	Research Chemist
	Bruce Citron	Senior Staff Fellow
		LNC NIMH
		LNC NIMH
		LNC NIMH
	Jennifer Tipper	Senior Staff Fellow
	Lisa Stekol	Biologist
	Robin Pruett	Visiting Student
		LNC NIMH
		LNC NIMH
		LNC NIMH
COOPERATING UNITS (if any) John Donlon, University of Galway, Ireland; J. F. Gusella, Neurogenetics Lab, Massachusetts General Hospital		
LAB/BRANCH Laboratory of Neurochemistry		
SECTION		
INSTITUTE AND LOCATION ADAMHA, NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.3	2.3	2.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>High level (25% of total protein) expression of a phenylalanine hydroxylase sequence lacking the first seven amino acid residues has been achieved in <u>E. coli</u>. A wild type version of this clone is under construction to allow comparisons of catalytic properties and directed mutagenesis.</p> <p>Partial human phenylalanine hydroxylase cDNA clones have been characterized and full length clones are being pursued through the synthesis of optimized cDNA libraries.</p> <p>Expanded experiments with rats have been conducted where chemical induction of diabetes in rats has been performed in conjunction with insulin treatment to clearly define the mechanism of increased phenylalanine hydroxylase activity and mRNA in diabetic rats.</p> <p>A full length coding sequence for human dihydropteridine reductase has been expressed on a bacterial vector and enzyme isolated from large scale cultures has been characterized.</p> <p>The dihydropteridine reductase clone has been used as a probe to identify the location of this gene on chromosome 4 near the Huntington's Disease locus.</p>		

Project Description:

Through a combined, interdisciplinary approach, we are studying the structure-function relationships of the aromatic amino acid hydroxylases and related enzymes to explain the precise biochemical mechanisms involved in the reactions which these enzymes catalyze. Importantly, with the aid of molecular biology, we can more readily modify these enzymes in very specific ways to clearly define the molecular interactions required for proper catalysis. We also plan to use cDNA clones of these enzymes as probes to study aspects of the molecular genetics of certain neurological and psychiatric disorders.

Major Findings:

Phenylalanine Hydroxylase:

A rat phenylalanine hydroxylase cDNA clone (missing only the N-terminal seven amino acids) was subcloned into a prokaryotic high level expression system and active enzyme was synthesized as the predominant (25% of total) protein in those cells. Oligomers coding for the N-terminal residues have been synthesized and the initial digestions and ligations to produce a copy of the wild-type, full length protein have been completed.

Many human phenylalanine hydroxylase cDNA clones have been isolated but none of them proved to be full length. cDNA to human liver poly(A)⁺ mRNA has been synthesized in preparation for packaging into a lambda vector so that a complete coding sequence may be found.

Phenylalanine hydroxylase activity and mRNA expression have been studied in broader experiments with diabetic rats to characterize certain aspects of hormonal regulation involving this enzyme. In these studies, we plan to demonstrate the reversibility of the observable effects by the use of insulin.

Tyrosine Hydroxylase and Tryptophan Hydroxylase:

We have been screening various cDNA libraries for functional copies of these genes. We have human and rabbit brain tissues rich in these enzymes from which we have been synthesizing cDNA to create libraries to isolate these clones.

Dihydropteridine Reductase:

We have subcloned a full length cDNA of the human enzyme into an E. coli expression vector (achieving levels of approximately 1% of total crude protein). Purified protein from a large scale (300 l) culture of these cells is under study.

This clone was radioactively labeled and used to map the DHPR locus on human chromosome 4 (at a site near the Huntington's Disease locus) and identify RFLPs which could be used in screening variant alleles of this gene.

6-pyruvoyl-tetrahydropterin synthase:

Antibody to this enzyme was used to begin screening cDNA libraries for clones of this gene. Two putative clones have been isolated from the immunoscreening.

Significance to Biomedical Research and Proposed Course:

Phenylalanine Hydroxylase

Phenylketonuria results from an untreated deficiency of phenylalanine hydroxylase. A major symptom of this disease is severe, irreversible mental retardation. This indicates that individuals carrying a mutant allele that yields a partially deficient enzyme may have some propensity for mental disorders. We will be using cDNA probes to characterize alterations in messenger RNA levels that have biological effects and site directed mutagenesis will allow us to elucidate the functional elements of this protein.

Tyrosine Hydroxylase

This enzyme is the primary control point for the neurotransmitter pathway implicated in several diseases such as Parkinsonism. Recent findings have been consistent with at least one type of mutation in this gene being responsible for at least one type of manic depressive disorder. Using cDNA probes, we will study the transcriptional response of a wild-type copy of this gene in neuroblastoma cells to a wide variety of stimuli. These probes will also be used to characterize the modifications in tyrosine hydroxylase that lead to some of the diseases explainable by altered catecholamine metabolism.

Tryptophan Hydroxylase

The neurotransmitter, serotonin, is an end product of the metabolism of tryptophan by tryptophan hydroxylase. Decreased activities of all three aromatic amino acid hydroxylases produce pronounced central nervous system disorders broader than the symptoms due to any characterized or postulated abnormality of any one hydroxylase. Conversely, there is a large array of mental diseases for which no biological explanation exists. Aberrant tryptophan hydroxylase expression might be responsible for some of these disease states and we will attempt to identify these cases using hybridization probes.

Dihydropteridine Reductase (DHPR) and 6-pyruvoyl-tetrahydropterin synthase (PTS).

All three aromatic amino acid hydroxylases have an absolute requirement for the cofactor, tetrahydrobiopterin. DHPR and PTS are involved in the production of this cofactor and individuals deficient in one of these activities have atypical phenylketonuria. This is a severe disease involving mental retardation, CNS problems, movement disorders, etc. Several patients have been identified having marked deficiencies in each of these enzymes. We

will first characterize the genetic basis in the known cases and also use these probes to identify changes in less severe and less obvious classes of such disorders.

Publications:

1. MacDonald ME, Anderson MA, Lockyer JL, Milstien S, Hobbs WJ, Faryniarz AG, Kaufman S, Ledley FD, Woo SLC and Gusella JF Physical and genetic localization of quinonoid dihydropteridine reductase gene (QDPR) on short arm of chromosome 4. Somatic Cell and Molecular Genetics 1987;13:569-74.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01092-10 LNP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Frontal Lobe and the Cerebral Control of Behavior

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Steven P. Wise Research Biologist LNP, NIMH

Others: Shraga Hocherman Visiting Associate LNP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH Animal Center, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

1.7

PROFESSIONAL:

1.7

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The primate frontal lobe consists of three main parts: the primary motor cortex (MI), the prefrontal cortex (PF), and the nonprimary motor cortex. The nonprimary motor cortex can be divided into at least two fields: the supplementary motor cortex (SM) and the premotor cortex (PM). Previous work on this project has clarified the definition of these cortical fields by an analysis of (a) neuronal responses to peripheral inputs, (b) thresholds for evoking movements with intra-cortical electrical stimulation, (c) the properties of single neurons during the performance of several visuomotor tasks, and (d) cytoarchitectonics. After preliminary definition of the areas, we used the techniques of behavioral neurophysiology, developed in the LNP, to study the functional organization of the caudal frontal lobe in primates. Our results support the hypothesis that, among their functions, PM and SM play a role in the preparation for future action. Many individual research projects have been designed and successfully executed to rule out alternatives to motor preparation (or 'set') as the functional correlate some activity we observe in PM and SM. However, we have recently found that not all PM activity is linked to motor functions. A study of "anticipatory discharges" in PM indicates that neuronal activity pattern does not reflect the preparation of movement and instead appears to play a role in anticipating the timing and/or nature of events.

Objectives:

The goal of this project is to gain a better understanding of functional organization of the primate frontal lobe and to contribute to the development of a unified hypothesis of its role in directing behavior. Our approach to an analysis of frontal lobe mechanisms consists of a multidisciplinary experimental strategy involving the formulation and testing of hypotheses with the techniques of behavioral neurophysiology.

Methods:

Each one of ten subprojects is described below:

(1) One monkey was operantly conditioned to depress one of four keys located in a perimeter at arms length. While the monkey pressed one key, another of the four keys, selected randomly, was illuminated after a randomly varied delay period. This key thereby became the next target. An auditory cue near the target could be substituted for illumination of the target. A barely discernable visual cue near the target key, appearing after another variable delay, signaled the monkey to move and depress the target. The monkey was required to make the movement within a short period of time, near the limit of reaction time. The purpose of this subproject was to make an initial survey of neuronal activity patterns in PM in order to help define it and gain insight into its physiological organization. Electrical stimulation and cytoarchitectonic techniques were also used. Another important aspect of this subproject was a comparison of neuronal activity when visual vs. auditory signals instruct the monkey to make the same movement.

(2) Two monkeys were conditioned to align two spots of light on a screen. One of these spots was controlled by a computer (the target spot), the other by the arm movements of the animal (the position spot). The monkey was required to align the spots within a small accuracy "window." In five-sixths of the trials, after a short period of time, the target spot jumped to one of six locations. The monkey had to maintain his arm position unchanged until the target spot dimmed, at which point it was required that the monkey flex or extend his forearm rapidly and accurately in order to realign the position spot with the target spot. In one-sixth of the trials, the computer selected a situation in which physically identical stimuli signaled the animal to make no movement. This experiment was designed for two purposes: to contrast neuronal activity in MI and PM and to distinguish neuronal activity when identical stimuli signalled the execution vs. the withholding of movement.

(3) Two monkeys were operantly conditioned to depress the central of three keys located on a panel at arm's reach. After a period of time, either the left or right key became illuminated. Three experimental conditions ensued: (a) the left or right key remained illuminated and served as the target for the subsequently triggered movement, (b) the light was turned off before the monkey was allowed to execute the movement, forcing the monkey to remember the proper target, or (c) the target light was switched before the monkey was allowed to execute the movement. This experiment was designed to test the competing hypotheses that PM set-related activity reflects a continued visual stimulus and that

it reflects the preparation for movement. In addition, we could compare cell activity when the monkey performed the task described above and a self-paced movement between the left and right keys.

(4) One monkey was conditioned to execute a single limb movement as well as a short sequence of two limb movements in the same direction. The monkey was seated in front of a panel of three keys as in subproject 3. Each trial started with the monkey pressing the leftmost of the three keys. Two experimental conditions ensued: (a) the central key was illuminated, thus indicating that a single movement was to be made, or (b) both the central and right lights were simultaneously illuminated to indicate that a short motor sequence was to be initiated. This subproject was designed to test the hypothesis that PM is especially important in guiding simple sequences of movement.

(5) Two monkeys were conditioned to respond to two different sorts of visuospatial instruction signals. The first type of instruction signal was comparable to that described in the subprojects described above, i.e., the visual cue itself was "directional"; indeed it was incorporated within the target, a touch pad located at arm's length. The second type of instruction was one in which the instruction cues contained no directional information: a blue lamp meant to move the limb to the right and a yellow lamp to move to the left. Thus, the relationship of the stimulus to the movement in the latter situation was "abstract" or "arbitrary". This subproject tested the following hypothesis: if PM activity reflects the preparation for movements, there should be little or no difference in activity in the two situations.

(6) Two monkeys were conditioned to make the same movements under two different experimental conditions: (a) when visual instructions of the type described for subprojects 1, 3 and 5 guided the movement, and (b) when the identical cues were irrelevant or nonexistent and the monkey guided its behavior via internal (i.e., nonsensory) processes. This study was designed to test the hypothesis that PM is especially important when sensory signals instruct a movement and to contrast activity in PM with that in SM, which has been hypothesized to play a special role when internal processes instruct a movement (P.E. Roland et al, J. Neurophysiol., 1980, 43: 118; J.C. Eccles, Arch. Psychiat. Nervenkr., 1982, 231: 423; G. Goldberg, Behav. Brain Sci., 1985, 8: 567).

(7) Two monkeys were trained to respond to one visual stimulus with a hand movement and to another visual stimulus with a foot movement. The cell activity in PM before hand movements was compared to that before foot movements. Before either movement, the monkey was required to withhold movement for a variable delay period. This study was designed to examine the internal organization of the premotor cortex, in particular whether it had topographic pattern of organization.

(8) The activity of cells in PM of one monkey were compared in two experimental conditions: (a) when a visual cue instructed the monkey about where the target of the next limb movement should be, as well as when to execute the movement, and (b) when a visual cue indicated to the monkey only when to execute the movement. In condition b, the monkey had no environmental cue upon which to base its choice of two potential targets. Instead, the monkey simply had to

guess and was prevented from adopting the strategy of moving always to one target (thereby receiving rewards on half the trials) by an algorithm that randomly selected what target would be rewarded. That algorithm decreased the probability that a movement to a given target would be rewarded by 0.1 every time the monkey made as many as three consecutive movements to that target. This study was designed to study a pattern of neural activity we had previously discovered in PM (Mauritz and Wise, 1986), which we termed anticipatory activity. We wanted to know whether anticipatory activity reflected the preparation for movement long in advance, or, as an alternative, played a role in nonmotor functions like the prediction of the timing and/or nature of partially predictable environmental events.

(9) The activity of PM and SM neurons in two monkeys was studied when the monkey made straight, simple arm-projection movements to a target vs. a variety of curved, complex movements to the same target. The monkey was seated in front of a bit-pad, which was beneath a plexiglass panel composed of 14 light-emitting diodes. A set of 2 or 3 diodes was illuminated briefly to instruct the monkey about the trajectory of the movement to be executed on that trial. There were three different possible targets on each behavioral trial and three different trajectories to each target: one simple and straight, the other two curved and complex. This subproject was designed to test the hypothesis that PM and/or SM are specialized for the control of complex movement trajectories, an idea originally proposed by Moll and Kuypers (Science 198: 317-319, 1977) on the basis of cortical ablation effects.

(10) The activity of neurons in PM and the dorsolateral prefrontal cortex (PF) were compared in one monkey under two experimental conditions: (a) an initial instructional cue, a centrally located green or red light, indicated the direction in which the next movement should be made, and (b) the identical stimulus was presented, but did not indicate the next rewarded movement direction. Instead, in the latter condition, the monkey had to remember the central stimulus (red or green) and await a second stimulus consisting of a light to the left and a second light to the right. In all cases one of those lights was red and the other one was green. The monkey had to respond in the direction of the cue of the same color as that previously presented centrally. The purpose of this experiment was to compare PF and PM activity when the monkey was preparing a movement vs. anticipating and preparing to receive a sensory input.

Major Findings:

1. Comparison of Primary and Nonprimary Motor Cortex. SM neurons were found to be much less responsive to peripheral somatosensory inputs than MI neurons in the same monkeys. The lack of profound somatic sensory responsiveness in these parts of the somatic sensorimotor cortex supports the hypothesis that SM plays its most significant roles in the guidance of movement by internal rather than proprioceptive processes. MI seems to be specialized for control of movement, in part, by cutaneous and noncutaneous mechanoreceptors that serve to adjust motor commands to compensate for internal or external events causing deviations from the animal's intended movements.

2. Cortical Field Definition and Internal Organization of Nonprimary Motor Cortex. Our findings have enabled us to improve the current understanding of

cerebral localization and functional specialization in the agranular frontal cortex (SM, PM, plus MI) and certain adjacent cortical field. Of special importance has been the effort to determine anatomical correlates of physiologically defined cortical regions. Microelectrode methods revealed that the boundary between MI and SM corresponds to the boundary between two anatomically defined parts of the agranular frontal cortex (termed areas 4 and 6). This differed from the accepted published maps at the time this study was undertaken. This line of inquiry has been taken up and elaborated as a separate project (Z01 MH 01096-04 LNP). Similar work has clarified the location of the boundary between PM and MI, and this work, too, is being elaborated in the separate project noted above. In the present project we have shown that PM can be distinguished from the MI representation by its markedly increased threshold for evoking movements with intracortical microstimulation. As for the internal organization, that of SM was studied in Z01 MH 01096-04 LNP, and that of PM was shown by the distribution of neurons with activity related to hindlimb vs. forelimb movement (subproject 7) to support previous indications that PM is topographically organized (K. F. Muakkassa, and P. L. Strick, Brain Res., 1979, 177: 176; K. Kurata, K. Okano and J. Tanji, Exp. Brain Res., 1985, 60: 188).

3. Premotor Cortex Physiology. Of most interest to us is the substantial population of neurons (about one-third of the cells in PM) that change their activity in relation to motor set. We have termed this pattern of activity "set-related", and we hypothesized that it is specifically correlated with the motor preparation of an animal (subproject 1). This hypothesis has been supported in several ways: (a) set-related units show changes in activity when visual signals cue a movement, thus establishing a specific motor set, but not when the same signals instruct the monkey to withhold movement (subproject 2); (b) if the visual instruction changes (to establish a different motor set), the unit activity rapidly changes to reflect the new set (subproject 3); (c) when the instruction is removed (but the set remains the same), the unit activity continues to reflect the set rather than the sensory signals (subproject 3); (d) the set-related activity before the first of a series of two movement is the same as that before the same movement when it is executed by itself (subproject 4); (e) set-related activity is usually the same when directional (left or right) instruction stimuli and arbitrary (yellow or blue) instruction stimuli instruct the same movement (subproject 5); and (f) set-related activity is usually the same when the monkey plans a movement on the basis of trial-specific visual stimuli as when the monkey plans the same movement on the basis of the memory of recent events (subprojects 6 and 8). In addition, it has been found that these and other premotor cortex units change their activity in advance of predictable environmental events. Such activity, which we call anticipatory activity, reflects or contributes to anticipating the timing and/or nature of temporally predictable events (subprojects 2, 3, 6 and 8), rather than the preparation for or execution of movement (subproject 8). Taken together, our findings improve the understanding of the set-related processes of PM and accord with the hypothesis that PM plays an important role in behaviors in which a movement must be retrieved from memory on the basis of highly flexible, arbitrary cues. These ideas are also being explored as part of Z01 MH 01096-04 LNP.

The past year has seen the virtual completion of data analysis on subprojects 5, 6, 7, and 8. We published a full-length paper on subproject 5 in Experimental Brain Research, and certain results of subproject 6 have been accepted for

publication in the same journal. The results of subproject 8 have been accepted for publication in the journal Somatosensory and Motor Research and the results of subproject 7, as well as further analysis based on subprojects 5 and 6, are currently being prepared for submission to first-rate journals.

Significance to Biomedical Research and to the Program of the Institute:

Studies of functional localization in higher-order motor cortical fields, such as the premotor cortex and supplementary motor cortex, are important to understanding the cortical control of motor acts of the least automatic kind, in both health and disease, and especially for understanding the ways in which sensory signals are converted, by the brain, into organized motor acts. A much improved knowledge of the nonprimary areas of the cerebral cortex and their relation to higher-order control of motor behavior may yield insight into higher brain functions of all types. More generally, much of this project is devoted to basic study of the frontal cortex, a presumed site of higher brain functions like reason, attention, forethought, and perception, as well as fine motor capabilities. Advances in this area are of fundamental importance in understanding the roles of the frontal lobe in mental health and disease.

Proposed Course of the Project:

The two main foci of this project, currently, are: (1) an explicit comparison of the physiological activity in PF and PM (subproject 10), and (2) a continuation of our study of the premotor cortex (PM) and its role in the selection and control of behavior (subproject 9).

We are contemplating two future initiatives for the present project. In the first, we plan to examine frontal cortex neuronal activity to determine the location of neurons subserving sensory memories and those subserving analogous representations of motor memories. The experimental design to achieve our objective can be summarized as follows: the monkey must learn to associate a color (there must be two) in a certain location in space (there must be four) with a specific motor act (there must be two). In one condition, the monkey is compelled to remember the sensory stimulus to be able, after a delay period, to execute the correct behavior. In the other condition, the monkey must remember the motor act associated with the visuospatial stimulus. It will be time consuming to condition monkeys to perform this difficult task, but we believe that it will be a very rewarding study if successfully completed. The distinction being tested, that between the sensory and the motor, between input and output, between perception and action, between memories and the behavioral uses of those memories, is one of the most important problems in cortical neurobiology. In the second initiative, we are completing a study of neuronal activity during ethologically more meaningful behaviors. We hope to develop telemetric methods to explore the activity of neurons in freely moving animals. This will enable us to study neuronal activity associated with vocalization when they are triggered in different ways. One way to trigger a vocalization in some monkeys is to isolate them from their social group. Following such isolation, they spontaneously trigger the motor act, i.e., the vocalization. Alternatively, the behavior can be brought under stimulus control, either via arbitrary, operantly

conditioned stimulus-response associations or response to recorded natural vocalization of conspecifics. We propose to examine the hypothesis that separate neuronal pathways subserve triggering of a behavior by these different conditions and stimuli.

Publications:

Kurata K, Wise SP. Premotor cortex of rhesus monkeys: Set-related activity during two conditional motor tasks, *Experimental Brain Research* 1988;69:327-343.

Kurata K, Wise SP. Premotor and supplementary motor cortex in rhesus monkeys: neuronal activity during externally- and internally-instructed motor tasks, *Experimental Brain Research* 1988 (in press).

Vaadia E, Kurata K, Wise SP. Neuronal activity preceding directional and non-directional cues in the premotor cortex of rhesus monkeys. *Somatosensory and Motor Research* 1988 (in press).

Wiesendanger M, Wise SP. Current issues concerning the functional organization of the primate motor cortex. In: Chauvel P, Delgado-Escueta AV, Halgren E, Bancaud J, eds. *Frontal Lobe Seizures and Epilepsies*. 1988 (in press).

Wise SP. The motor cortex. In: Adelman G, ed. *The Encyclopedia of Neuroscience*. Springer-Verlag, 1987;697-699.

Wise SP, Godschalk M. Functional fractionation of frontal fields. *Trends in Neuroscience* 1987;10:449-450.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01096-04 LNP
PERIOD COVERED October 1, 1987 through September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Spatial Organization of the Primate Motor Cortex		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P. I.:	Andrew R. Mitz	Senior Staff Fellow LNP, NIMH
Others:	Steven P. Wise Moshe Godschalk	Research Biologist LNP, NIMH Professor, Department of Anatomy Erasmus University, Rotterdam The Netherlands
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neurophysiology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH Animal Center, Poolesville, Maryland 20337		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>The purpose of this project is to examine the internal organizations and the inter-relationship among motor areas of the frontal lobe, including the <u>primary motor cortex</u> (SM), the <u>frontal eye fields</u> (FEF), the <u>supplementary eye fields</u> (SEF) and the <u>premotor cortex</u> (PM). The model species chosen for study is the rhesus monkey, because these motor areas have been best characterized in this species.</p> <p>In the first part of this project <u>intracortical microstimulation</u> is being employed to study the efferent <u>topography</u> of the cortical areas listed above. For the second part of the project <u>single-unit recording</u> is being employed to examine the possible involvement of the <u>premotor cortex</u> in learning new stimulus-response relationships. The goal of the first part is to understand the organization of the motor outputs of each cortical area. The second part will contribute to the understanding of the cortical motor areas in recall and execution of learned motor behaviors.</p>		

Objectives:

Movement-related single-unit activity in the primary motor area (MI), supplementary motor area (SM), premotor area (PM), the supplementary eye field (SEF) and frontal eye field (FEF) suggest that these cortical regions are involved in the programming and execution of movement. Anatomical evidence further suggests that these areas are extensively interconnected, and we hypothesize that these cortical fields act together to generate appropriate motor commands. The objective of this project is to first explore the topographic organization of these motor areas and then correlate this organization with aspects of their cytoarchitectonics. The second goal is to characterize and distinguish each area's contribution to descending motor commands.

Methods Employed:

1. Microstimulation mapping of cortical motor areas. Although intracortical microstimulation in awake or lightly sedated animals is a standard method for examining motor topography in MI and FEF, a modification of this technique was necessary to explore the somatotopy of SM and SEF.

The first phase of this project included developing a modified intracortical stimulation technique. This technique utilizes platinum-iridium electrodes with about 1000 μm of metal exposed at the tip, compared to standard microelectrodes with exposed tips of 100-250 μm . In addition to the electrode geometry, careful selection of stimulus parameters and the use of biphasic current pulses has contributed to successful SM and SEF mapping.

Rhesus monkeys were implanted with stainless steel chambers over SM, PM, FEF, and SEF. Electrodes were inserted through the dura and electrical stimuli were delivered periodically as the electrode descended through the cortex. The stimulus parameters were 0.2 ms duration or each phase of the biphasic pulses, 330 pulses/second, 31-pulse trains, and 65 μA search currents. Movements evoked at each stimulation site were identified by two observers and recorded.

2. Oculometry. In some instances eye movements were visually observed and recorded manually. Later, eye movements were recorded as electro-oculograms via implanted silver/silver-chloride electrodes or non-invasively with infrared oculometry. When the latter techniques were used, two channel directional information (left-right and up-down) was either recorded on separate magnetic tape channels or combined as an X-Y vector on a storage oscilloscope and photographed.

3. Quantitative cytoarchitectonics. The boundary between cytoarchitectonic areas 4 and 6 were first estimated qualitatively. Then, a computerized cell plotting system connected to a high power microscope was used to locate, identify, and measure individual cells on a series of histological sections. Cell body areas were measured in 21 sections separated by about 500 μm , and covered a 10 mm rostrocaudal extent. Cell measurements were made from an area 1 mm in dorsoventral extent and centered midway between the dorsal surface of the hemisphere and the cingulate sulcus. Each cell body greater than 20 μm in any dimension was circumscribed under 400 X magnification. The coordinates of the

boundary were recorded by the computer, which then computed the area of the cell body. Any stained portion of the proximal dendrites was included in the cell area measurement.

4. Chronic single-unit recordings. A novel behavioral paradigm is being used to evaluate PM, SM, and MI unit activity before and after stimulus-response associations are learned. New hypotheses concerning the functions of PM suggest that one of its roles is to recall an appropriate motor program based upon recognition of a stimulus. To study the validity of this approach, monkeys have been trained to move a lever to one of 3 positions in response to color figures. The set of color figures are changed periodically to restart the learning process. Single-units are being studied as the animal learns which figure matches which target position. Unit activity will also be recorded during performance of well-established movement responses to familiar figures. The hypothesis will be supported if the response of a PM unit changes as a new figure-target relationship is established, and if the final pattern of activity associated with the new figure resembles that during responses to the well-known figure.

Major Findings:

1. Although early surface electrical stimulation studies demonstrated motor topography within SM, more recent attempts to verify this topography with microstimulation and other methods have failed. The modified microstimulation method developed for the present study has yielded a topographic map of motor organization. In this map, orofacial movements, including movement of the pinnae, lips, tongue, and jaw, and conjugate eye movements to the contralateral visual hemifield, were observed most rostrally. Adjacent to and overlapping with this orofacial and eye movement region was a region from which forelimb movements were evoked. Evoked forelimb movements usually included action at 2 or more joints. Caudal to and overlapping the sites evoking forelimb movement was a third region of the SM from which hindlimb and tail movements were evoked. The hindlimb and tail region of SM merged with the hindlimb region of MI without a distinguishable boundary. The overall rostrocaudal extent of the SM covered 12-14 mm. This arrangement agrees with the general organizational pattern originally observed with surface stimulation, but also demonstrates that SM has a less discrete topography than presumed by many earlier investigators.

The region of frontal cortex over which saccadic eye movements can be evoked with low stimulus currents had at one time been thought to be limited to the FEF. Based on our recent results and those of others (Schlag and Schlag-Rey, J. Neurophysiology, 1987, 57:179), it has been shown that a second eye movement field is located in or immediately rostral to SM. This field has been termed the supplementary eye field (SEF). Using the new stimulation technique, we have evoked eye movements from sites along a thin strip of cortex extending from the FEF to the SEF. The SEF was found to be broader in cortical extent than described in the literature, and it extends to within a few millimeters of SM. Despite their proximity to each other, movements evoked from the FEF were exclusively 'vector' saccades of a fixed displacement and direction, regardless of initial ocular position. Within the SEF, however, evoked eye movements included a mixture of vector and 'goal-directed' saccades. Goal-directed saccades (Schlag and Schlag-Rey, 1987) always terminated near the same places regardless of initial orbital position.

Preliminary simulation in and around the arcuate sulcus indicates that forelimb movements can be reliably evoked from PM with our modified microstimulation method. Forelimb movement sites have been identified deep in the arcuate sulcus adjacent to the FEF.

2. Quantitation of cell sizes along the medial wall of the cortex has allowed an objective evaluation of the cytoarchitectonic transition from area 4 to area 6. The rostrocaudal distribution of cell bodies greater than $600\text{ }\mu\text{m}$ and of cell bodies greater than $1200\text{ }\mu\text{m}$ show that these populations decrease from a peak to low densities over a 4 to 5 mm range. Neurons with areas of $300\text{--}500\text{ }\mu\text{m}$ do not have the same rostrocaudal change in density. The qualitatively-determined boundary between area 6 and area 4 corresponds to the rostrocaudal level at which the largest pyramidal cells appear, at more caudal levels, in substantial densities. The location of this boundary determined relative to the tail representation (of MI, SM, or both) agrees well with that described previously. These findings show that cytoarchitectonic boundaries can be identified with quantitative methods and confirm the correspondence of SM with the medial aspect of area 6.

3. The single-unit study described above has been started. Results from this study must await further data collection and analyses, but they appear to be promising.

Significance to Biomedical Research and to the Program of the Institute:

The project represents part of a larger effort to explore the physiological and anatomical organization of the frontal cortex. Examining the internal organization of PM, FEF, SEF, and SM, and their interactions with the other frontal areas is of importance in gaining an overall understanding of the frontal cortex and its role in health and disease.

Proposed Course of the Project:

The current project involves a systematic study of frontal motor areas, MI, SM, FEF, SEF, and PM. Before the start of this project, the somatotopy of MI had been well established. Improvements to the standard technique of microstimulation has allowed the demonstration of SM topography, as well as a reassessment of the topography of eye movements in the FEF and SEF.

There are many remaining questions about PM organization, including the nature of its topography, if any, the number and spatial organization of functionally discrete fields, and how these fields operate together with the other motor areas of the cortex and basal ganglia to orchestrate movement.

An important step in answering these questions is to complete PM stimulation mapping. It is also valuable to pursue, in parallel, our study on the role of PM in motor learning.

Further work on PM topography will depend upon the outcome of the current preliminary mapping study. This study was started in the Laboratory of Neurophysiology with Dr. Godschalk, a Fogarty Visiting Associate. The study will continue in Dr. Godschalk's laboratory at Erasmus University in Rotterdam, The

Netherlands, as a joint project. If the results from stimulation show that the PM forelimb representation resides between the FEF and the forelimb representation of MI, then the mapping work will be completed. If, however, the maps contradict current ideas concerning PM organization, the stimulation mapping can be combined with an anatomical tracer study. In such a study either the forelimb or the hindlimb representation of PM and MI will be elucidated with electrical stimulation mapping and then tracer dyes will be injected into each representation. This combination anatomical and physiological methods to study PM topography in macaque monkeys made is possible by the new stimulation method described above.

While the PM mapping is continued on a joint basis in Holland, the single-unit recording experiment will be conducted at the NIHM. Finding cell activity changes during learning of a visuomotor association will demonstrate that PM has a central role in the generation of learned movements. Additional experiments could then be undertaken to understand the transition from the unlearned to the learned condition. If units in the PM are insensitive to the difference between novel stimuli and those associated with a specific motor response, then current interpretation of PM lesion experiments (by others) must be re-examined.

Publications:

Mitz AR and Wise SP. The somatotopic organization of the supplementary motor area: Intracortical microstimulation mapping. J. Neuroscience 1987;7:1010-1020.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01097-02 LNP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Activity of Corticostriatal Neurons in Motor Cortex of Primates During Wrist Movement

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Melvyn P. Heyes	Visiting Associate	LNP, NIMH
Others:	Steven P. Wise	Research Biologist	LNP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH Animal Center, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|--------------------------------------|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

DISCONTINUED

Principal Investigator transferred to the Laboratory of Clinical Science.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01098-02 LNP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Anatomical Analysis of Neuronal Circuits

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Chisato Asanuma Stanfield Senior Staff Fellow

LNP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH Animal Center, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The structural organization of the thalamic reticular nucleus was investigated by injecting the fluorescent dye, Lucifer yellow, intracellularly into identified neurons in fixed tissue slices of the rat brain. This method provides a complete picture of the dendritic geometry of reticular nucleus neurons and is particularly useful for exploring the microcircuitry of selected regions of the brain, since it can be used in conjunction with other neuroanatomical techniques. Having successfully established the facilities for this work, the structure of individual neurons in the thalamic reticular nucleus has been carefully examined and documented. The afferent input to the thalamic reticular nucleus has then been studied using both anterograde and retrograde axonal fiber tracing techniques. In addition to the well known inputs from collaterals of principal neurons in the thalamus and corticothalamic neurons in layer VI of the cerebral cortex, the general region of the reticular nucleus also receives inputs from several cholinergic cell groups. These include the parabrachial nucleus, the pedunculopontine nucleus, and the magnocellular nucleus basalis. Anterograde tracer injections into the parabrachial nucleus or the pedunculopontine nucleus label axonal arbors within the closely adjacent zona incerta, but do not label those within the reticular nucleus. In contrast, tracer injections into the nucleus basalis result in a significant amount of diffusely organized and widespread axonal terminal arbors within the thalamic reticular nucleus. These findings suggest that the thalamic reticular nucleus may be pivotal in the cholinergic modulation of inputs to the cerebral cortex.

OBJECTIVES:

The aim of this project is to elucidate, at the single-cell level, the structural details of the dendritic arborizations of identified neurons and the exact locations of identified afferent axonal arbors upon them. In particular, we seek to: (a) characterize the dendritic morphology of thalamic reticular nucleus neurons; (b) define the afferent inputs to this thalamic nucleus, and (c) to identify the details of the relationship between (a) and (b). These issues are of interest since the thalamus occupies a pivotal position in the transmission of virtually all extrinsic information entering the cerebral cortex and since recent studies have shown that the thalamic reticular nucleus is inhibitory and directly influences the principal relay neurons of the thalamus.

METHODS:

This investigation involves the intracellular injection of the fluorescent dye, Lucifer yellow, into identified neurons in the brain, in conjunction with anterograde and retrograde neuroanatomical labeling techniques.

Injections of neuroanatomical tracers are made into various brain foci in rats. Following appropriate survival periods, the rats are perfused with heparinized saline, followed by a buffered aldehyde fixative solution and the brains sectioned on a vibratome or on a freezing microtome. Selected regions are examined microscopically using both low power objectives and high power oil immersion objectives, and documented with photomicrographs or with camera lucida drawings.

In those experiments involving the intracellular Lucifer yellow injections, the brains are sectioned on a vibratome, counterstained with nuclear yellow, secured on glass slides with a nitrocellulose membrane filter, and examined using a fluorescent microscope, equipped with a high power (40x) water immersion objective, with 1.6mm working distance. Neurons in the thalamic reticular nucleus are identified and impaled under visual guidance with glass microelectrodes introduced at an angle of 10-30 degrees from horizontal, and containing a 2% solution of Lucifer yellow. As the dye is iontophoresed into the neurons, it quickly diffuses throughout their dendritic arbors. Following the injection procedure, the sections are removed from the membrane filter, and, when appropriate, run through standard immunohistochemical procedures. The sections are then mounted on slides and prepared for microscopic examination and documentation as briefly outlined above.

MAJOR FINDINGS:

In the interpretation of neuronal circuitry of the thalamic reticular nucleus, the structure of the postsynaptic elements, or the neuronal dendrites, has been based largely on results obtained using the century-old Golgi techniques. Unfortunately, Golgi studies have predominantly relied upon data generated in young animals (since the Golgi stain does not work effectively in this thalamic region of older animals). Recent studies of the development of several neuronal systems indicate that rather major structural changes take place during the immediate postnatal period in the structure of both axons and dendrites. Thus, many anatomical based on Golgi methods need to be reexamined with other methods in adult animals. The Lucifer yellow injection procedure is less capricious than Golgi methods, and works quite effectively in adult animals.

Thalamic reticular nucleus neurons are large multipolar neurons, usually with elongated somata, whose diameters range from 25 - 50 μ m in the longer dimension. Their dendritic

arborizations are quite distinctive. The dendritic arborizations of these neuron are planar, and occupy one or two tiers of restricted thickness; each dendritic arbor occupies a thin tier of less than 10 μm , and radiates extensively within the thicker sheet like plane of the nucleus, which averages about 200 μm in width. Each cell body has 3-5 primary dendrites, which rapidly give rise to 2-4 secondary dendrites. The primary dendrites are smooth, while secondary and tertiary dendrites are beaded; the multitudes of filamentous spine-like appendages, previously reported in the rat with the Golgi technique, are rarely seen. Many dendritic processes extend over 250 μm in length.

To investigate the afferent inputs upon these neurons, a series of experiments were done, in which several retrograde tracers were injected into the region of the thalamic reticular nucleus. The tracers used were fast blue, WGA-HRP, or fluoro gold, and the results were similar with each tracer. Following such injections, retrogradely labelled neurons occur predominantly ipsilaterally in the parabrachial nucleus, the pedunculo pontine nucleus, and in the caudal magnocellular nucleus basalis (Ch. 4), in addition to neurons in the locus coeruleus, dorsal nucleus of the raphé, dorsal thalamus, and layer VI of the cerebral cortex.

Recent immunohistochemical studies have established that high concentrations of choline acetyl transferase and somatostatinergic immunoreactivity are present within axonal profiles in the thalamic reticular nucleus. The precise origin of these immunoreactive terminal processes has, however, been uncertain. We have, accordingly, made anterograde tracer (WGA-HRP and PHA-L) injections into the parabrachial nucleus, the pedunculo pontine nucleus, and into the magnocellular nucleus basalis. WGA-HRP and PHA-L injections into the parabrachial nucleus or the pedunculo pontine nucleus label axonal arbors within the zona incerta, closely adjacent to the thalamic reticular nucleus, but does not significantly label axons within the thalamic reticular nucleus. PHA-L injections into the magnocellular nucleus basalis, however, show extensive and widespread terminal arbors within the thalamic reticular nucleus. Thin, beaded axons, arborize extensively within the thalamic reticular nucleus, and individual axonal processes can frequently be followed for distances of up to 2 millimeters.

In a preliminary series of experiments, the newly introduced carbocyanine dye, D-282 (Dyl) was injected into the frontal cortex of a series of rats. This fluorescent dye is reported to label a small population of axons anterogradely, and has the advantage over the PHA-L procedure for labeling axonal arbors in that it can be immediately visualized upon sectioning, and does not require immunohistochemical treatment. With the use of this anterograde tracer, therefore, the identification and selection of the postsynaptic cells to be injected and filled with Lucifer yellow could be significantly expedited. In these preliminary experiments, 5 - 10 labeled axons were observed to emanate from the injection sites in the cerebral cortex, join the internal capsule, and subsequently curve posteriorly to enter the dorsal thalamus. Each axon generally gives off one collateral branch that leaves the parent stem axon at a right angle while traversing the thalamic reticular nucleus, and which arborizes extensively within the reticular nucleus.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE:

The reticular nucleus of the thalamus has for long been held to be the final link in the ascending 'non-specific' activating system mediating the desynchronization of the cortical EEG (i.e. arousal) (see eg. Hanbery et al., *Electroenceph. Clin. Neurophysiol.*, 1954, 6:103). The results of more recent studies indicate, however, that this nucleus may, instead, be a part of a circuit that modulates the transmission of signals through specific thalamic relay nuclei. The thalamic reticular nucleus receives an input from collaterals of thalamocortical neurons, and an input from collaterals of corticothalamic neurons. These inputs have been demonstrated to be topographically very specific, with each part of the reticular nucleus receiving inputs from corresponding

portions of the dorsal thalamus and the cerebral cortex, and projecting back to topographically appropriate nuclei in the dorsal thalamus. Since the dorsal thalamus occupies a pivotal position in the transmission of information to the cerebral cortex, the functions of the thalamic reticular nucleus has recently been the subject of much speculation. It has been postulated to be involved in the modulation of the perceived amplitude of externally generated stimuli (Ahlsén et al., Exp. Brain Res., 1985, 58:134), and in the control of selective attention (Skinner and Yingling, Prog. Clin. Neurophysiol., 1977, 1:30; Crick, Proc. Natl. Acad. Sci., 1984, 81:4586). Others have, however, maintained that the thalamic reticular nucleus mediates general levels of arousal (Steriade et al., J. Neurosci., 1986, 6:68).

Of particular interest has been the recent immunohistochemical demonstrations indicating that the thalamic reticular nucleus contains significant levels of gamma-amino butyric acid (GABA) and somatostatin in its neurons (Houser et al., Brain Res., 1980, 200: 341; Graybiel and Elde, J. Nsci., 1983, 3: 1308), and choline acetyl transferase in axonal profiles (see eg. Levey et al., J.C.N., 1987, 257:317). Since neither the reticularis neurons, the thalamocortical neurons nor the corticothalamic neurons are likely to be cholinergic, the exact source of these cholinergic axonal profiles has been an enigma. The positive demonstration of a significant, yet diffusely organized input to the thalamic reticular nucleus from the magnocellular basal nucleus, which is well established to be cholinergic, indicates that this thalamic nucleus does indeed receive an input which may account for its immunoreactive axonal profiles, and that this input is organized in a way which is capable of modulating the excitability of neurons in this thalamic nucleus and of the thalamus in general. The magnocellular basal nucleus neurons are, of course, well known to degenerate in Alzheimer's disease. Thus it is possible that some of the more general symptoms, such as the disturbances of sleep, levels of arousal, and perception of external stimuli common in patients with Alzheimer's dementia may, in part, be due to a degeneration of inputs to the thalamic reticular nucleus from the magnocellular basal nucleus. Furthermore, since these global functions are also frequently impaired in various mental disorders, a detailed examination of the circuitry modulating the transmission of inputs from the periphery to the cerebral cortex is an important step towards a further understanding of many mental health disorders.

PROPOSED COURSE OF THE PROJECT:

The thrust of this project over this coming year will be to identify the exact relation between each population of afferent axonal arbors with the dendritic profiles of thalamic reticular nucleus neurons. A detailed understanding of the interrelation between the various axonal arbors with their postsynaptic targets in the thalamic reticular nucleus should provide important clues concerning the role of the thalamic reticular nucleus in the transmission of signals from subcortical centers to the cortex.

In particular, we hope to:

- (1) Identify the exact relationship between axonal arbors originating in the magnocellular nucleus basalis with the dendrites of thalamic reticular nucleus neurons.
- (2) Determine whether the PHA-L labeled axonal arbors originating in the magnocellular nucleus basalis are cholinergic, as hypothesized.
- (3) Determine whether the same neurons in the basal forebrain that project to the thalamic reticular nucleus also project to the cerebral cortex.

- (4) Examine the thalamocortical axon collaterals and the corticothalamic axon collaterals in the thalamic reticular nucleus in detail, and study their relationships with the dendrites of thalamic reticular nucleus neurons.

PUBLICATIONS:

Asanuma C., Ohkawa R., Stanfield BB, and Cowan WM, Observations on the development of certain ascending inputs to the thalamus in rats. I. Postnatal development. *Developmental Brain Research* 1988 (in press).

Asanuma, C., The functional organization of the mammalian dorsal thalamus: Anatomy of the primate ventral lateral complex. In: Stein, B.M., and Holtzman, R.N.N., eds. *Contemporary Perspectives in Neurosurgery; Surgery of the Diencephalon*. New York: Plenum 1988 (in press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01099-02 LNP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurochemical Interactions Between Cortical and Striatal Dopaminergic Activity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Melvyn P. Heyes	Visiting Associate	LNP, NIMH
Others:	Elisabeth Murray	Senior Staff Fellow	LN, NIMH
	Jan Johannessen	Senior Staff Fellow	LCS, NIMH
	Stephen Suomi	Chief	LCE, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH Animal Center, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Disturbances in the balance of regional brain dopaminergic activity have been implicated in the symptomatology of schizophrenia. Recent studies in patients have led to the hypothesis that dysfunction in the dorsolateral prefrontal cortex (DLPFC) may cause the impaired motivation, shallow affect, and deficiencies in the performance of problem solving tasks suffered by schizophrenics. Analogous deficits are produced in primates by depletion of dopamine from the DLPFC. Together these observations suggest a role for DLPFC dopaminergic neurons in cognitive functions subserved by this area. Studies in rats have indicated that dopaminergic activity in the medial frontal cortex exerts a suppressive effect of dopaminergic activity in the striatum and nucleus accumbens. Weinberger has postulated that decreased dopaminergic activity in the DLPFC in schizophrenia may increase dopaminergic activity in striatum resulting in the positive features of schizophrenia. To determine whether a reciprocal relationship between prefrontal and sub-cortical dopaminergic activity exists in primates, dopamine was depleted in juvenile rhesus monkeys by local injections of 6-hydroxydopamine. Neurochemical analysis indicated that dopaminergic activity was increased in the putamen.

Objectives:

Studies in rodents indicate that dopaminergic activity in the frontal cortex influences dopaminergic activity in striatum and nucleus accumbens. Specifically, Carter and Pycock reported that depletion of DA in the median prefrontal cortex (MPFC) of rats, by a local injection of 6-hydroxydopamine (6-OHDA), increased DA in nucleus accumbens (*J. Neurochem.* 34: 91-99, 1980; *Br. Res.* 192: 163-176, 1980). In a later study, DOPAC and HVA concentrations were also increased in nucleus accumbens (*Nature* 286: 74-77, 1980). Bannon and Roth (*Pharmacol. Rev.* 35: 53-68, 1983) and Weinberger and co-workers (*Arch. Gen. Psychiatr.* 43: 114-124, 1986) have linked these apparent reciprocal relationships between cortical and sub-cortical dopaminergic activities to the symptoms of schizophrenia. Specifically, negative features of schizophrenia result from decreased dopaminergic activity in the dorsolateral prefrontal cortex (DLPFC) and the positive features of schizophrenia results from the concomitant increase in dopaminergic activity in striatum. Application of rodent studies to primates is often tenuous because neuro-anatomical and neurochemical structures in brain are not necessarily homologous. The DLPFC in primates is considered a homologue of the MPFC in rodents.

Because of the prominence of the studies of Carter and Pycock and the pathogenic interpretation of their data, we began studies on the effects of depletion of dopamine from the dorsolateral prefrontal cortex (MPFC) in rhesus monkeys. Two control and two DLPFC-lesioned monkeys were studied in the preliminary study. The project is a collaboration with Dr. E. Murray, NIMH, Dr. J. Johannessen, NIMH and Dr. S. Suomi, NICHD.

Major Findings:

Local injections of 6-OHDA throughout the DLPFC depleted dopamine and DOPAC and increased HVA in DLPFC and orbitofrontal cortex. In the putamen, dopamine and 3-MT were increased, DOPAC slightly decreased and HVA unchanged. However, the ratio of dopamine to each metabolite was increased. In caudate nucleus, dopamine and HVA concentrations were increased but there were no changes in the ratio of dopamine to metabolites.

REGION	DLPFC	CHANGE IN CATECHOLAMINE: >10% CONTROL		
		ORBITO- FRONTAL	PUTAMEN	CAUDATE
CATECHOLAMINE:				
<u>Dopamine</u>	-98 %	-62 %	+52 %	+31 %
<u>DOPAC</u>	-74 %	-31 %	-14 %	0
<u>HVA</u>	+24 %	+55 %	0	+19 %
<u>3-MT</u>	-----	-----	+20 %	0
RATIOS:				
<u>Dopamine/DOPAC</u>	-----	-----	+85 %	0
<u>Dopamine/HVA</u>	-----	-----	+46 %	0
<u>Dopamine/3-MT</u>	-----	-----	+27 %	0

Significance to Biomedical Research and to the Program of the Institute:

The results obtained so far have extended a hypothesis concerning the mechanisms responsible for schizophrenia to primates and have brought us closer to developing an animal model of the disease. We have found indications of increased dopaminergic activity in the putamen of rhesus monkeys following damage to the frontal lobes.

Proposed Course of the Project:

Because the preliminary results were obtained in two control and two dopamine-lesioned monkeys, we intend increasing the number of animals in each group to enable statistically meaningful analysis to be done. A number of further biochemical tests will also be done including the characteristics of regional dopamine receptors and the activities of catecholamine metabolizing enzymes in key brain regions.

Publications:

None.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02250-04 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Purification of Messenger RNAs Encoding for Neurotrophic Factors in the Rat Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Anne-Marie Duchemin, M.D., Visiting Associate, Neuropsychiatry Branch, IRP, NIMH

Dr. Thanh Tam Quach, Visiting Associate, NICHD, NIH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH; Dr. Bruce K. Schrier, Laboratory of Developmental Neurobiology, NICHD, NIH

COOPERATING UNITS (if any)

Laboratory of Developmental Neurobiology, NICHD, NIH, Bethesda, MD.

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Aging

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

.60

PROFESSIONAL:

.60

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Brain injury is associated with morphological, immunological, and biochemical modifications, some of which may be involved with a healing process. Neurotrophic factors have been shown to appear in the rat brain after lesion. This project intends the molecular cloning of one of the genes encoding for these lesion-induced neurotrophic factors.

A cDNA library has been constructed from a mRNA fraction from lesioned rat brain, which was shown to be capable of inducing neurotrophic activity when injected into *Xenopus* oocytes.

Screening of the library by differential colony hybridization and by the test of the neurotrophic activity of the fusion protein of the clones has lead to the isolation of several clones. DNA sequencing and analysis of the fusion-protein of these clones is in progress.

Project Description:

Objectives: Neurotrophic and neurite-promoting factors increase in the brain after injury (possibly to facilitate repair), decrease secondary neuronal death, and increase reactive sprouting of undamaged axons. These factors are at highest concentration in the area surrounding the lesion and peak 1-2 weeks after the lesion. They support survival and neurite outgrowth of peripheral and central neurons in culture and facilitate graft survival.

Because of the limited amount of source tissue and the instability of the proteins that hamper their purification, we attempted to clone the cDNA encoding for one of these neurotrophic factors. These factors could be useful for increasing the functional effects of brain tissue transplantations. A major obstacle to clinical applications of brain grafts is their limited efficacy. The availability of neurotrophic factors could enhance growth of the grafts.

The purpose of this project was the purification of one of the neurotrophic factors that seem to be produced in the brain after injury. These factors could be useful for increasing the functional effects of brain tissue transplantations. A major obstacle to clinical applications of brain grafts is their limited efficacy. The availability of neurotrophic factors could enhance growth of the grafts.

Methods Employed:

(A) Techniques for assaying neurotrophic activity: Neurotrophic activity is assayed on cell cultures of sympathetic neurons from 12-day-old chicken embryos. Miniaturization of the assay was introduced to increase the level of detection for neurotrophic factors.

(B) Source of neurotrophic factor: We used injured brains of rats as a source of neurotrophic factors. The periphery of a vacuum aspiration wound of the cortex was removed 7 days after lesion and was used as a source of neurotrophic factors and of corresponding messenger RNA.

(C) In vivo translation in *Xenopus laevis* oocytes: *Xenopus laevis* oocytes have been used for an in vivo translation of mRNA into proteins. After fractionation of mRNA from lesioned rat brain on a sucrose gradient, mRNA fractions were injected into oocytes and the translation product tested for neurotrophic activity.

(D) Molecular cloning: Different techniques have been used: cDNA synthesis, hybridization-subtraction, cloning in Bluescribe M13 and PGEM blue vectors, colony hybridization, transcription, Northern and Southern blot, protein gel electrophoresis, DNA restriction mapping, and single and double stranded DNA sequencing.

Major Findings: In vivo translation into oocytes has allowed us to select a fraction of mRNA that seemed to encode for a neurotrophic factor.

A cDNA library has been constructed from this mRNA fraction in the vector pGEM blue. Twenty thousand colonies have been screened by differential colony hybridization with cDNA probes from mRNA of control rat brain and from mRNA of lesioned rat brain. The clones that seemed to be induced or showed a clear

increase in lesioned brain were grown individually and the fusion protein synthesis induced by IPTG. Fusion proteins were assayed by pools and then individually in serial dilutions on the chicken sympathetic ganglion neuron survival assay.

Two of the clones showing some neurotrophic or neurite-promoting activity were sequenced. DNA sequencing of the first revealed a large homology with mitochondrial ribosomal RNA. Analysis of the fusion-protein directly on a protein gel or analysis of the *in vitro* translation product of this clone showed the synthesis of a 45-kilodalton protein. The physiological role of this protein remains to be determined.

A second clone with a fusion protein demonstrating neurite-promoting activity was shown by analysis of the DNA sequence to be a portion of the cytochrome oxidase gene. Three other positive clones are being sequenced, and the library is being screened with the clones from a specific lesioned-rat brain library constructed by subtraction in the vector bluescribe M 13.

Significance to Mental Health Research: We look forward to obtaining data from this project that may be applied to the understanding and treatment of the degenerative diseases of the CNS and recovery of patients with brain injury.

Proposed Course of Project: Analysis of the fusion-proteins and DNA sequencing of the positive clones from the library screening will be pursued.

Results from the *in vivo* translation of neurotrophic factors in *Xenopus* oocytes have been analyzed for publication.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02252-04 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Behavioral Pharmacology and Toxicology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

William J. Freed, Ph.D., Chief, Preclinical Neurosciences Section, NPB, IRP, NIMH

Dr. Dale E. Braun, Naval Hospital

COOPERATING UNITS (if any)

Department of Neurosurgery, Naval Hospital, Bethesda, MD.

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Preclinical Neurosciences

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.2

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The project on behavioral pharmacology and toxicology is aimed at elucidating the neuropharmacological processes involved in abnormal brain function and the development of techniques to intervene in abnormal brain function through pharmacological manipulations. The studies involve the induction of abnormal behavioral and physiological states through administration of drugs and other manipulations; they attempt to alter behavior and physiological states by pharmacological manipulations. Particular topics of interest are areas known to be associated with the actions of neuroleptic drugs and drug-induced psychoses. These include (1) phencyclidine, which in humans can induce abnormalities resembling schizophrenia; (2) calcitonin and calcium-channel inhibitors and activators, under investigation because of possible relationships between calcium mechanisms and long-term changes in neuronal function induced by chronic neuroleptics; (3) seizures and other phenomena related to the quisqualate type of glutamate receptors, of particular interest because of the close association between glutamate synapses and dopamine synapses on striatal neurons; and (4) changes in various neuronal systems, particularly excitatory amino acid systems induced by chronic neuroleptic administration. The latter is being investigated to enhance understanding of the mechanisms of neuroleptics' action.

Project Description:

Objectives: This research program is aimed at development of: (1) animal models of brain dysfunction, (2) arrests induced by pharmacological agents, and (3) pharmacological agents that alleviate the behavioral manifestations of these manipulations.

Methods Employed: Behavioral studies include observations of seizures, tests of maze-learning and operant conditioning, and measurements of rotational behavior, feeding and drinking behavior, and general locomotor activity. Animals will also be subject to the induction of brain lesions through administration of drugs and neurotoxins and stereotaxic injection of neurotoxic substances. Histological studies are also performed for some experiments.

A. Behavioral Pharmacology of Phencyclidine

Objectives: This research program is aimed at development of animal models to measure behavioral responses to phencyclidine (PCP), employ these models to assess various agent's ability to antagonize the behavioral effects of PCP, and characterize the behavioral pharmacology of PCP. The experiment's immediate purpose is development of pharmacological agents to treat adverse reactions of PCP abuse. The long-range goal is development of agents to treat schizophrenia and/or elucidate causes of schizophrenia.

Methods Employed: Behavioral observations and testing for general locomotor activity following systemic or intracerebral administration of PCP.

Major Past Findings: PCP is considered to be one of the best pharmacological models of schizophrenia because of the wide range of psychological reactions to its abuse. The reactions include sensory disturbances (rather than vivid visual hallucinations, as with LSD) and activation similar to that produced by amphetamine; violent and even prolonged psychotic reactions are common. In initial studies, a large series of pharmacological agents was screened for their ability to block PCP reactions in mice. Only a few agents, including phenothiazine neuroleptics, GABA agonists, and yohimbine and methysergide, were effective behavioral antagonists of PCP. Other agents were ineffective, including some that are used clinically, such as diazepam and haloperidol. In a subsequent study, a variety of neuroleptics were tested for their ability to antagonize PCP. Significant differences among neuroleptics were found: methiothepin and fluphenazine, phenothiazines in general were most effective; haloperidol, pimozide, molindone, and sulpiride were relatively ineffective.

A study of the genetics of PCP reactions in recombinant inbred strains of mice has also been performed. This was done to develop animal models of severe vs. mild PCP reactions in humans and to determine whether reactivity to PCP is determined and unrelated to responsivity to amphetamine. BALB-strain mice showed pronounced reactions to PCP while C57 B1/6-strain mice reacted much less markedly (about one-third as much locomotor stimulation). Most of the recombinant strains showed intermediate reactions. BALB-strain mice may, therefore, provide a good model for severe PCP reaction in humans. Both BALB- and C57 B1/6-strain mice were found to be similarly susceptible to blockade of PCP-induced stimulation by haloperidol.

New Findings: No new studies this year.

Proposed Course of Subproject A: Studies of new neurotransmitter antagonists or agents with suspected neuroleptic activity may be tested as antagonists of PCP-induced stimulation as well as amphetamine-induced stimulation—if warranted by other studies in the Neuropsychiatry Branch.

B. Seizures and Amino Acids

The excitatory amino acids glutamate and aspartate and their decarboxylated inhibitory counterparts GABA and glycine are major and ubiquitous putative regulators of neuronal excitation and inhibition. Excitatory neurotransmitters potentially may be involved in neuropsychiatric disorders in two distinct ways. First, disturbances in amino acid neurotransmitter function may be directly involved in epilepsy, and possibly involved in schizophrenia. Schizophrenia is exacerbated by administration of amino acids such as methionine, and there is some evidence that methionine's effects are due to metabolic conversion to the excitatory substance homocysteine. The disease homocystinuria is also accompanied by behavioral disturbances. Second, there is increasing evidence that neuronal injury caused by excess stimulation by excitatory amino acids, such as glutamate or kainic acid, is a major mechanism of CNS neurotoxicity, and has been hypothesized to be involved in Huntington's chorea and in the neuronal damage consequent to ischemia. Thus, there is a potential involvement of excitatory amino acid toxicity in schizophrenia and other neuropsychiatric disorders as well.

Methods Employed: These studies are conducted primarily by administration of drugs either systemically or intracerebrally into the lateral ventricles, followed by observation and blind scoring of seizures. Seizures are induced by chemical agents, auditory stimulation, or electrical stimulation of the brain. Current studies also involve induction of seizures by administration of amino acid agonists, such as quisqualate, directly into the cerebral ventricles. Some experiments involve administration of excitatory substances to mice on neonatal day 1, followed by measurement of weight gain and histological assessment of neuronal damage.

Major Past Findings: A considerable body of literature in the past has shown that schizophrenia can be exacerbated by administering the amino acid methionine. We obtained evidence from animal studies that some of the effects of methionine appeared to be due to accumulation of its metabolite homocysteine rather than to increases in methylation reactions, as was originally supposed. Evidence has also been obtained that homocysteine is a relatively specific agonist of the quisqualate-sensitive or "Type II" excitatory amino acid receptor site. Glutamic acid diethyl ester (GDEE), a quisqualate antagonist, also antagonized homocysteine-induced seizures. Betaine, which is involved in the remethylation of homocysteine, has also been found to have anticonvulsant properties. This anticonvulsant effect is mediated by the central nervous system, and appears to be a pharmacological effect, rather than a metabolic effect because its metabolically inactive metabolites sarcosine and dimethylglycine have effects similar to those of betaine. Homocystinuria, a disorder involving excess accumulation of homocysteine, related amino acids, mental retardation, and seizures, has been reported by others to be successfully treated with betaine. This area is also of interest as induction of brain damage by excessive excitatory amino acids has recently been recognized as a major neurotoxicological process and could be involved in the genesis of developmentally related brain abnormalities.

A model for the induction of seizures by the direct intracerebral administration of quisqualic acid was developed. These seizures were found to be blocked by systemic GDEE. Quisqualate-induced seizures were blocked by valproic acid, but most other anticonvulsant drugs had no effect. Diazepam partially antagonized quisqualate-induced seizures in high dosages. These data are consistent with the hypothesis that the quisqualate-sensitive receptor is involved in some forms of seizure phenomena, particularly those which can specifically be blocked by valproic acid.

New Findings:

1. Structure-activity studies suggest that deaminated derivatives of GDEE possess anticonvulsant activity equal to or greater than that of GDEE. Increasing carbon chain lengths by more than one over that of GDEE resulted in decreased activity. Methyl esters were less potent than ethyl esters.
2. GDEE was examined for induction of ataxia as a general measure of toxicity. Whereas most other anticonvulsants and other types of excitatory amino acid antagonists induced severe ataxia in dosages about two-fold larger than the anticonvulsant dosages, GDEE did not produce ataxia at any dosage tested. Although GDEE is not very potent on a mg/kg basis, these data suggest that more potent derivatives of GDEE might have useful anticonvulsant activity.
3. A model for inducing seizures by the direct activation of central calcium channels with the calcium-channel agonist BAY K-8644 has been developed. Activation of central calcium channels can induce seizures that can be altered by chronic treatment with calcium channel blockers. More recently, the methods for evaluation of BAY K-8644-induced seizures have been revised to separate several different components of the seizure phenomena. BAY K-8644-induced seizures have been found to be extremely resistant to anticonvulsants, but easily blocked by calcium channel antagonists. Drugs that alter calcium metabolism, such as chlorpromazine, alter but do not block BAY K-8644-induced seizures. This model of calcium-related epileptogenesis may help to elucidate the relative role of calcium in the actions of various convulsant and anticonvulsant drugs.

Proposed Course of Subproject B: The concept of inducing seizures by the direct intracerebral application of specific agents with known actions may result in the development of a set of seizure models, each with known specific properties. It may, therefore, become possible to examine pharmacological agents with potential antiseizure activity against this set of models and to develop a more accurate preclinical pharmacological characterization than is generally obtained by global models such as pentylenetetrazol and electroconvulsive shock-induced seizures. Moreover, the methods and information obtained in these studies are further applied in studies of chronic neuroleptic effects described under Subproject E: Chronic Neuroleptic Studies.

C. Developmental Arrest

Developmental arrest consists of brief interference with development of the central nervous system, usually induced by the administration of short-acting antimetabolic agents during critical periods of brain development. Such models can be employed to produce diffuse but relatively restricted brain abnormalities,

such as reductions in cortical thickness or volume of the striatum. Administration of the antimitotic agent methylazoxymethanol (MAM) to rats on the 15th day of gestation, for example, interferes with cortical development and induces behavioral hyperactivity, putative deficits in learning, and hyperinnervation of the cerebral cortex by catecholaminergic fibers. The potential relevance of this model to neuropsychiatric disorders has prompted us to conduct additional studies.

Major Past Findings: Developmental arrest induced by MAM serves as a model of abnormal development due to brief interference with CNS growth. When administered during growth of the cortex, the cerebral cortex does not fully develop, leading to a variety of behavioral abnormalities. This model may parallel some of the minor abnormalities of CNS structure recently reported in schizophrenia. A concomitant of this model is an excessively dense catecholaminergic innervation of the cerebral cortex.

We have previously measured a variety of behavioral indices in animals with developmental arrest induced by MAM. Essentially all of the observed abnormalities could be explained by the hyperactivity present in the animals treated by MAM. For example, learning abnormalities were present only when the learning tasks required increased behavioral output for successful performance.

New Findings: No new studies this year.

Course of Subproject C: As information about CNS pathology and brain atrophy in schizophrenia continues to develop, attempts to produce a developmental arrest model of schizophrenia may receive additional impetus. For example, the MAM model may be applied to test for effects of chronic neuroleptics on cortico-striatal system (see Subproject E).

D. Calcitonin

Calcitonin, a peptide hormone secreted by the C-cells in the thyroid, is primarily involved in regulation of peripheral calcium metabolism. Our group has found that calcitonin is also, however, a very potent inhibitor of eating behavior, and this effect is mediated directly by the brain. Calcitonin has subsequently been reported to inhibit amphetamine-induced stimulatory effects. Even though calcitonin is not produced by the brain it may be an important peripherally derived hormonal regulator of behavioral processes through actions on the central nervous system.

Methods Employed: Using standard procedures, animals receive chronic cannula implants into various brain nuclei or into the lateral ventricle. Calcitonin or other substances are then administered through the cannulae in small amounts, and eating behavior, activity, and other behavioral responses are measured. In some experiments, the animals also receive systemic injections of d-amphetamine or calcitonin.

Major Past Findings: We have previously found that calcitonin is an extremely potent inhibitor of eating behavior in animals; this effect has been found to be mediated via the CNS. Others have reported that calcitonin is capable of inhibiting amphetamine-induced behavioral stimulation. There is some evidence that calcitonin is a hormone that serves to modulate calcium uptake by certain CNS neurons.

The specific site of action of calcitonin within the CNS has been localized by measuring behavioral responses to calcitonin following local intracerebral injections through chronically implanted cannulae. Responses to calcitonin were obtained from hypothalamic nuclei, especially the paraventricular nucleus, the perifornical area, the supraoptic nucleus, ventromedial nucleus, and nucleus reuniens. Responses were also obtained from the vertical limit of the diagonal band and from the nucleus accumbens. The peripheral effects of calcitonin are known to diminish with age. We found, however, that the CNS effects did not decrease with age, suggesting that the CNS effects and peripheral effects are independent.

The intracerebral localization of the antagonism of amphetamine-induced hyperactivity by calcitonin has been examined. Calcitonin was found to inhibit amphetamine-induced activity when administered directly into several hypothalamic areas, including the paraventricular nucleus, the perifornical area, the floor of the hypothalamus (anterior), the lateral hypothalamus, and the nucleus accumbens and zona incerta.

The finding that calcitonin inhibited eating when administered into the floor by the hypothalamus was further examined to determine whether areas involved in regulating behavior are located in this region. Calcitonin, neurotension, bombesin, and cholecystokinin were locally administered into the hypothalamus floor, and the animals were examined for changes in eating behavior and activity. Cholecystokinin had no effect anywhere it was infused. Each of the other peptides was found to have potent behavioral effect in some parts of the floor; the effect differed for each of the peptides. These data suggest that some parts of the floor of the hypothalamus have an important role in behavioral regulation.

New Findings: No new studies this year.

Proposed Course of Subproject D: Studies are planned to further investigate effects of calcitonin and calcitonin analogues on stimulant drugs (including amphetamine, apomorphine, and PCP) with differing mechanisms of action.

E. Chronic Neuroleptic Studies

One of the most salient properties of neuroleptics is the gradual onset of antipsychotic effects after several weeks of treatment. Because the antipsychotic effect is delayed, neuroleptics' mode of action cannot be entirely understood solely by acute studies. So, a series of experiments on changes in responsiveness to various pharmacological agents and brain neurotransmitter receptors following chronic drug treatment has been initiated. These studies may also be relevant to mechanisms for induction of tardive dyskinesia.

Major Past Findings: Previous studies have shown alterations in responsiveness to GABAergic and cholinergic agents following chronic neuroleptic administration. Recent anatomical investigations by other groups have shown an intimate association between the glutamate-mediated corticostriatal and the dopamine-mediated nigrostriatal pathways. The possibility of interactive changes involving these two systems is therefore being investigated.

New Studies: Experiments to evaluate behavioral responsiveness to glutamate agonists and antagonists after chronic neuroleptic treatment have shown that chronic neuroleptic (haloperidol) administration attenuates the behavioral

effects of quisqualic acid, a glutamate agonist. The seizures induced by quisqualate were not altered. The effects of glutamate diethyl ester, a glutamate antagonist, were also attenuated by chronic haloperidol treatment. Additional experiments to evaluate striatal glutamate receptors following chronic neuroleptic administration are starting, as the laboratory space for this project has just become available.

Proposed Course of Subproject E: The course of these experiments will be re-evaluated upon completion of the experiments now in progress. Studies of neuronal membrane function and studies in human post-mortem material may also be considered.

Significance to Mental Health Research: This program attempts to investigate some possible forms of pharmacological intervention in animal models of schizophrenia. These data are of value to mental health research in three ways. First, basic information about relationships among brain neurochemical systems is obtained. Second, these data may suggest possibilities for clinical treatment protocols. Finally, preclinical data useful for applying clinical protocols are sometimes obtained.

Proposed Course of Behavioral Pharmacology and Toxicology Project: This project is expected to continue indefinitely, as an interactive process with clinical sections of the branch.

Publications:

Freed WJ. Non-specific postjunctional supersensitivity and the therapeutic latency for the antipsychotic effect of neuroleptic drugs, Schiz Bull, in press.

Freed WJ, Braun DE. Anticonvulsant activity of deaminated analogues of glutamic acid diethyl ester (GDEE), Brain Res, in press.

de Beaurepaire R, Freed WJ. Anatomical mapping of the rat hypothalamus for calcitonin-induced anorexia, Pharmacol Biochem Behav, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02253-04 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Tissue Transplantation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

William J. Freed, Ph.D., Chief, Preclinical Neurosciences Section, NPB, IRP, NIMH

Drs. Maciej Poltorak and Renaud de Beaurepaire, Visiting Associates, NPB, IRP, NIMH; Drs. Herbert Geller and Jeff Laskin, Rutgers University; Drs. Saul Schwarz, Sean Logan, and Dale E. Braun, Naval Hospital; Dr. Gary Simonds, Walter Reed Army Hospital; Dr. Jill Becker, University of Michigan

COOPERATING UNITS (if any)

Dept. of Pharmacology, Rutgers University, New Brunswick, NJ; Dept. of Neurosurgery, Naval Hospital, Bethesda, MD; Dept. of Neurosurgery, Walter Reed Hospital, Washington, D.C.; Dept. of Psychology, University of Michigan, Ann Arbor

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Preclinical Neurosciences

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

3.3

PROFESSIONAL:

0.8

OTHER:

2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study is primarily aimed at transplanting catecholamine-containing tissues, including adrenal medulla, tumor cells, and embryonic brain tissue, into the brain. Its purpose is to elucidate the properties of these tissues after transplantation and the response of the host brain to the transplanted tissues. Specifically, these experiments employ non-primate animal models to (1) develop the techniques of brain tissue transplantation for clinical use in Parkinson's disease; (2) develop brain tissue transplantation techniques so they eventually may be applicable to other disorders, such as schizophrenia and epilepsy, if and when these disorders become well enough understood to permit such applications; and (3) elucidate factors that control the development and the responses of the brain to injury or impairment, with particular emphasis on the nigrostriatal dopamine system. During the past reporting year, significant progress has been made in these areas.

Project Description:

Objectives: The overall objective of this study is to develop brain tissue transplantation as a technique for the repair of localized damage to the central nervous system. More specific objectives are to: (1) develop the techniques of brain tissue transplantation so they may be applied clinically to Parkinson's disease and to other disorders, such as schizophrenia or Alzheimer's disease if and when these disorders become well enough understood to permit such applications; and (2) employ brain tissue transplantation to elucidate the factors that control the development and plasticity of the brain, particularly the nigrostriatal dopamine system.

Methods Employed: The studies involve surgical, behavioral, and histological-histochemical procedures in animal subjects.

Major Past Findings: Grafts of embryonic substantia nigra or young adult adrenal medulla have been shown to decrease rotational behavior consequent to unilateral lesions of the substantia nigra (SN). The SN grafts produce dopamine, reinnervate the host caudate-putamen, and decrease spiroperidol binding in the striatum concomitant with their behavioral effects. The adrenal medulla grafts also produce dopamine, but do not reinnervate the host brain, apparently exerting their behavioral effects simply through secretion of catecholamines, followed by diffusion into the host brain tissue. Although these intracerebral grafts survive indefinitely across major histocompatibility typings, inducing rejection through peripheral sensitization of the host animals has been found to be possible. It should be emphasized that the behavioral effects of both adrenal medulla and SN grafts are relatively limited in magnitude. In general, the SN grafts appear to be limited in their efficacy because of a limited reinnervation of the host brain, while the effects of adrenal medulla grafts are limited because of limited survival of the grafted cells.

Intrastriatal adrenal medulla grafts have been found to survive indefinitely, albeit to a limited extent. These grafts produce some behavioral effects, particularly when obtained from young donors. Attempts at improving the performance of substantia nigra grafts have been initiated and are continuing. Substances such as gangliosides, haloperidol, and estrogen were found to have no effect on substantia nigra grafts.

1. **Trophic effects of cortical and striatal lesions on substantia nigra grafts:** These studies are intended to exploit the possibility of secretion of trophic substances by damaged brain tissue to enhance the penetration of dopamine-containing fibers from SN grafts into the host brain tissue. In an initial experiment, cortical lesions were found to increase the growth of fibers from grafts into the host brain, but only in the most dorsal part of the striatum, close to the lesioned brain area. Reinnervation of other parts of the striatum was not changed by lesions. Results of a long-term study also support this conclusion. These differences were not due to anatomical distortion of the brain from the lesions or to other anatomical artifacts. The cortical lesions themselves were also found to reduce rotational behavior by substantia nigra grafts. Another experiment, involving kainic acid lesions of the striatum, confirmed the stimulatory effect of brain lesions on reinnervation of the striatum by substantia nigra grafts. In contrast to cortical lesions, kainic acid lesions of the striatum tended to slightly enhance the behavioral effect of

grafts. Studies of cell survival in substantia nigra grafts and effects of other types of lesions are continuing.

2. Combined substantia nigra and striatal grafts: A study of the effects of combined grafts of substantia nigra and embryonic striatum into the lateral ventricle has been performed. The substantia nigra grafts were found to completely innervate the embryonic striatal grafts in preference to the host brain. When a striatal graft was present in the lateral ventricle, little or no innervation of the host striatum occurred. This study suggests that the mature denervated striatum is a relatively inferior target when compared to immature striatum. This study also suggests that the limited efficacy of substantia nigra grafts is due to properties of the target tissue rather than a limited efficacy of the substantia nigra grafts themselves.

3. Substantia nigra grafts in neonatal hosts: To exploit the favorable properties of embryonic striatum as target tissue for substantia nigra grafts, a paradigm was devised to transplant embryonic substantia nigra into the lateral of normal newborn rats within one day after birth. (Control animals received sciatic nerve grafts.) The animals were then allowed to grow to maturity, and received bilateral lesions of the substantia nigra. The presence of neonatally implanted substantia nigra grafts protected the animals against development of aphagia, adipsia, akinesia, and rigidity induced by the SN lesions. Differences between substantia nigra-grafted rats and controls were substantial. For example, rats with substantia nigra grafts were 3.7 times as active as the controls. Surviving grafts were consistently found to be well-incorporated into the host striatum. Therefore, the effectiveness of substantia nigra grafts can be increased by transplantation into neonatal hosts.

4. Trophic effects on intraparenchymal adrenal medulla grafts: Efforts have been directed at assessing trophic effects and implantation techniques for intraparenchymal grafts of adrenal medulla. Studies performed thus far include evaluation of the effects of co-implantation of adrenal medulla with tissues containing corticosteroids (adrenal cortex), nerve growth factor (NGF) (mouse submaxillary gland) or other unidentified trophic substances (rat iris); effects of prior lesions of the implantation site; assessment of graft survival in inbred rat strains (to rule out the possibility of partial rejection of the grafts); and the possible influence of trophic substances such as NGF. Some data have been decoded and found to be suggestive. These findings have not been conclusive. These studies are ongoing.

5. Development of a cat model: To have available another model for brain grafting techniques in higher-order species, we have taken steps to develop procedures for cats. So far, we have procedures for behavioral testing and for producing complete unilateral lesions of the substantia nigra. Three cats so far have received adrenal medulla grafts. No changes in behavior were produced by intraventricular grafts unless they were inserted into a cavity in the wall of the caudate nucleus. These studies are still in progress.

6. Chronic intrastratial catecholamine infusions: To determine the actual rate of catecholamine secretion required for adrenal medulla grafts to produce positive behavioral effects in animals with substantia nigra lesions, we have begun studies of chronic dopamine infusions using osmotic mini-pumps. Chronic dopamine infusions produce transient behavioral activation and stereotypy in both normal animals and those with unilateral substantia nigra lesions; the infusions

produce a longer lasting suppression of apomorphine-induced rotational behavior. Diffusion of the infused dopamine is localized to within 2-3 mm of the infusion site. Dose-response studies and additional characterization of these infusions are under way. Other studies include effects of intraventricular infusions and alternate buffer systems. It has been determined that intraventricular infusions are not effective.

7. Catecholamine release from brain grafts: In collaboration with Dr. Jill Becker of the University of Michigan, studies to measure catecholamine release from brain grafts using intracerebral dialysis probes have been initiated. The probes and a technique for implanting brain grafts through chronic cannulae have been developed. Data obtained so far suggest that catecholamines released from adrenal medulla grafts in the lateral ventricle are not "washed away" in the cerebrospinal fluid. (See section 9).

8. Effects of laminin and collagen on brain grafts: Studies of the effects of laminin (a basal lamina component that stimulates neurite growth) and collagen (a non-specific supporting substance) on growth of substantia nigra and adrenal medulla grafts in the ventricle have been undertaken. Results suggest that implantation of these grafts in these media does not alter their effects on rotational behavior.

9. Effects of adrenalectomy and adrenal cortex on intraventricular adrenal medulla grafts: Adrenal corticosteroids are known to influence the differentiation of adrenal chromaffin cells. Studies on the development of adrenal medulla grafts under conditions of varying steroid hormone concentrations have therefore been initiated. Results so far suggest that adrenalectomy may somewhat enhance the efficacy of adrenal medulla grafts. Although the overall difference between adrenalectomized and sham-operated host animals was not statistically significant, approximately 50% of the adrenalectomized hosts showed very large reductions in rotational behavior (between 80 and 90%), which was well outside the normal range and also more than is usually observed. No histological correlate of this difference has yet been detected. These data clearly suggest that the presence of the host adrenal gland is not necessary for behavioral efficacy of adrenal medulla grafts. The basis for the large effects in some of the adrenalectomized hosts is still under investigation.

One interesting finding was that blood concentrations of dopamine were strongly correlated to the degree of behavioral efficacy of these grafts. This correlation was present in all animals, but was particularly strong in the sham adrenalectomized data. It is possible, therefore, that adrenal medulla grafts work primarily by secreting catecholamines into blood vessels, and that these catecholamines are delivered to the host brain by virtue of leakiness in blood vessels adjacent to the graft. Additional factors might be operative as well, particularly in adrenalectomized host animals.

10. Effects of embryonic age of substantia nigra grafts: Recent studies of human embryonic substantia nigra grafts in rat hosts suggest that very early gestational tissue may be required for behavioral efficacy. These studies are inconclusive, however, because of immunological and possibly trophic differences between the rat hosts and the human donors. There has, surprisingly, been no direct study of optimal donor ages in a rat allograft model. To correct this oversight, we have investigated substantia nigra grafts from various ages of rat donors in rat host animals. Roughly similar effects were obtained with donors

from 11-17 days gestation, with 15-day gestational material being the most effective. The earliest material required longer periods to reach maximum efficacy. These data suggest that there is no particular gestational period at which substantia nigra must be obtained for behavioral efficacy, and that very early and relatively late material can both be effective.

11. Effects of adrenal medulla grafts in neonatal hosts: Using the same paradigm as for substantia nigra grafts, effects of adrenal medulla grafts when implanted into immature host animals on the first day after birth were investigated. These grafts had a slight protective effect against subsequent bilateral substantia nigra lesions, but this effect was much less substantial than that produced by embryonic substantia nigra grafts. These data suggest that the enhanced effects of substantia nigra grafts in neonatal animals are due to a specific interaction between embryonic substantia nigra and immature host brain.

12. Sensitization of host animals to established brain allografts: It has become fashionable to suggest that the so-called immunological privilege of brain does not exist or that brain grafts survive only because brain tissue is not immunogenic. Previous studies have shown that rejection of established brain allografts can be precipitated by peripheral skin grafts. This may, however, occur only because the generalized immune reaction to skin grafts induces MHC expression in the brain allografts, subsequently causing rejection of the skin grafts. To address this issue in another way, attempts were made to provoke rejection of established brain grafts by peripheral (subcutaneous) brain grafts and by peripheral administration of brain tissue with adjuvant. Results are not yet complete. Initial results suggest that peripheral brain and skin tissue produce roughly similar reactions to established brain grafts. Although the procedures employed so far have not produced rejection of brain grafts per se, definite immune reactions (consisting of lymphocyte infiltration and the appearance of large numbers of Ia antigen and helper T immunoreactive cells surrounding the grafts) is produced by peripheral brain grafts. These data so far are consistent with the possibility that brain grafts not only can succumb to an immune reaction, but can also provoke an immune reaction under appropriate circumstances.

13. Intraparenchymal transplantation of defined cell lines: Previous studies have found that PC12 pheochromocytoma cells survive intracerebral transplantation, in some cases for extended intervals. These cells were poor candidates for intracerebral grafting, however, because large aggregates of cells apparently induce extreme angiogenesis, resulting in graft rejection. Subsequent investigations have been directed at two cell lines known to possess the capacity to synthesize L-dopa, the B16/C3 melanoma, and N115E neuroblastoma cells. The B16/C3 melanoma cells apparently survive for extended periods and contain tyrosine hydroxylase, tyrosinase, and some catecholamines. These cells were not behaviorally effective. The N115E cell line also survives intracerebral transplantation and contains substantial amounts of tyrosine hydroxylase. Behavioral studies of N115E cell-grafted animals are not yet complete.

Significance to Mental Health Research: These studies may lead to the development of brain tissue transplantation as a therapeutic procedure for Parkinson's disease and eventually for other disorders. In addition, brain tissue transplantation is a valuable technique for the investigation of trophic functions in the brain. For example, the finding that brain injury has a trophic effect on dopamine-containing neurites is of potential importance for

understanding of the developmental and trophic influences on the brain's dopaminergic system and its possible dysfunction in schizophrenia. Subsequent studies using this paradigm may provide useful information relating to the effects of brain injury on neuronal circuits. Investigation of trophic functions and their possible absence is of particular importance for diseases such as schizophrenia, which may involve relatively subtle forms of neuronal dysfunction rather than readily detectable brain atrophy or neuronal degeneration.

Proposed Course of Project: Investigation of brain tissue transplantation as a therapeutic technique is expected to continue until a reasonably effective procedure applicable to Parkinson's disease is developed. Subsequently and concurrently, grafting will be studied primarily as a means of assessing trophic control of development and function of the brain dopaminergic systems. Studies of possible application of brain tissue transplantation to other disorders will also be continued in particular as developments in other fields elucidate possible applications.

Publications:

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de Beaufort R, Freed WJ. Anatomical mapping of the rat hypothalamus for calcitonin-induced anorexia, Pharmacol Biochem Behav 1987;127:177-82.

Shelton RC, Grebb JA, Freed WJ. Induction of seizures in mice by the calcium channel agonist Bay K8644, Brain Res 1987;402:399-402.

de Beaufort R, Freed WJ. Regional localization of the antagonism of amphetamine-induced hyperactivity by intracerebral calcitonin injections, Pharmacol Biochem Behav 1987;27:183-6.

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Adinolfi AM, Freed WJ. Neuronal plasticity: development and recovery from brain injury. In: Kaplan HI, Sadock BJ, eds. Comprehensive Text of Psychiatry, 5th ed. Baltimore: Williams and Wilkins, in press.

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Freed WJ, Dymek J, Poltorak M, Rodgers CR. Intraventricular brain allografts and xenografts: studies of survival and rejection with and without systemic sensitization. In: Sladek J, Gash D, eds. Progress in Brain Research, in press.

Becker JB, Freed WJ. Neurochemical correlates of behavioral changes following intraventricular adrenal medulla grafts: intraventricular microdialysis in freely moving rats. In: Sladek J, Gash D, eds. Progress in Brain Research, in press.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02255-04 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Calcium Channel Inhibitors: Interactions Systems - Human Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Gregory M. Straw, M.D., Medical Staff Fellow, Neuropsychiatry Branch, IRP, NIMH

Dr. Darrell Kirch, Senior Staff Fellow, NPB, IRP, NIMH; Dr. Llewellyn B. Bigelow, Associate Clinical Director for WAW Division, Saint Elizabeths Hospital; Dr. Edward Taylor, Clinical Social Worker, NPB, IRP, NIMH; Dr. Richard Suddath, Medical Staff Fellow, NPB, IRP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Preclinical Neurosciences

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

0.25

PROFESSIONAL:

0.25

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Calcium channel inhibitors (CCI) are thought to affect calcium flux through membrane-bound channels as their major site of action. Reports of clinical trials involving over 150 patients suggest that CCIs also have beneficial effects in neuropsychiatric disorders. Each of the 4 major subclasses of CCIs appears to have unique combinations of biochemical and behavioral properties. Additional studies have suggested a complex interaction between dopamine receptor function and calcium channels. We have completed a study of the clinical effects of verapamil in a schizophrenic population where trends toward improvement did not reach statistical significance. We have proceeded with the protocol to examine the effects of nifedipine in a similar cohort.

Project Description:

Objectives: To examine the clinical effect of nifedipine in a schizophrenic population.

Methods Employed: DSM-III-diagnosed patients will be followed with repeated neurological, neuropsychological, and psychiatric examinations as well as nursing BPRS.

Major Past Findings: Nifedipine-like drugs inhibited PCP-induced behavior in mice significantly better than did verapamil. Verapamil did not show statistically significant benefit in a cohort of schizophrenics.

New Findings: Nifedipine initially showed a trend to cause an overall worsening of psychotic symptoms but an overall improvement in abnormal movements. Final evaluation after 10 patients revealed no significant effect on psychotic symptoms, but a trend toward an improvement in dyskinetic movement in patients receiving active nifedipine.

Significance to Mental Health Research: There is a clear need for alternate medication for schizophreniform illnesses, and the CCIs may provide this alternate with a side effect profile significantly different from and possibly better than the current standards.

Proposed Course of Project: The project's data acquisition phase is complete, and the report is being compiled.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02256-04 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Defect Symptoms in Schizophrenia: Their Measurement, Correlates, and Treatment

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Darrell G. Kirch, M.D., Senior Staff Fellow, NPB, IRP, NIMH; Edward H. Taylor, Ph.D., NPB, IRP, NIMH

Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Renewed interest in the role of negative symptoms in "defect state" schizophrenia encouraged us to develop a "Negative Symptom Rating Scale (NSRS)" to more efficiently measure this syndrome. A study has been conducted performing a factor analysis of the NSRS. Studies are being conducted to explore the relationship between schizophrenia, social intelligence, general intelligence, negative symptoms, and premorbid social functioning in schizophrenic patients.

Project Description:

Objectives: Patients with schizophrenia are known to have social withdrawal, impaired social judgment, difficulty with problem solving, and defective motivation. This has been referred to as the "defect state" and these symptoms have been labeled "negative symptoms." The goals of this project are to characterize negative symptoms in patients with chronic schizophrenia using a rating instrument—the Negative Symptom Rating Scale (NSRS)—developed for that purpose within the laboratory. In turn, the NSRS is being used to evaluate the association between negative symptoms and impairments in standardized intelligence testing, social intelligence, movement disorders, and other clinical variables.

Methods Employed: Patients entering the research program of the Neuropsychiatry Branch are being evaluated using the NSRS. In addition, the Brief Psychiatric Rating Scale is used to evaluate more general psychiatric symptoms. Some patients are also rated using tests of social intelligence, social competence, and premorbid social functioning.

Testing of a large number of patients has facilitated the study of the internal consistency of the NSRS itself. In addition, correlations have been made between the NSRS and Brief Psychiatric Rating Scale, intelligence tests, social intelligence measures, and movement disorders as assessed using the Abnormal Involuntary Movement Scale.

Major Past Findings: The initial step in this project was publication of the 10-item NSRS, which provided a description of the scale and a method for performing ratings. The scale was found to be easy to administer, with acceptable inter-rater reliability, and was observed to have a high correlation between negative symptoms (as measured using the NSRS) and other previously published measures of negative symptoms. The scale itself was also used in a clinical trial involving the administration of vasopressin to patients with chronic schizophrenia. In that study, a modest but significant improvement was found in negative symptoms in the patients given vasopressin.

New Findings: In an attempt to examine the internal consistency of the NSRS, a factor analysis was conducted on data from NSRS evaluations of 121 patients with chronic schizophrenia. The results revealed a strong internal consistency for the scale, with a distribution of the 10 NSRS items into 2 factors. Moreover, defect signs and symptoms as measured by the NSRS appeared to occur independently of positive symptoms in this sample. In addition, when the defect state was examined in relation to the duration of illness in these patients, it appeared that the defect state increased in severity proportional with the duration of illness.

In a second study in which the relationship between defect symptoms and tardive dyskinesia was examined, no correlation was found between defect symptoms and tardive dyskinesia in 55 neuroleptic-treated, chronic schizophrenic patients. This study failed to replicate an earlier finding of increased negative symptoms in patients with tardive dyskinesia.

An additional project has demonstrated that the NSRS has a strong inverse relationship with current IQ as measured by the WAIS-R. This supports published assumptions by Crow and others that IQ decreases as negative symptoms increase.

The same study also found that the degree of defect state experienced by a patient is not dependent upon premorbid social functioning.

Significance to Mental Health Research: The defect state is a prominent part of the pathology of schizophrenia. The characterization of negative symptoms and an examination of their relationship to other clinical variables is important to our understanding of the disease as a whole. Moreover the identification of how social skills develop, are maintained, and become impaired has important implications for the treatment of schizophrenia.

Proposed Course of Project: The data regarding negative symptoms, general intelligence, and social intelligence will be gathered from a larger cohort of patients. These data will in turn be examined in relation to other clinical variables. Ultimately, this study will include correlations of NSRS data with other neuroscientific data gathered from patients in the Neuropsychiatry Branch research program. Content and construct validity studies of the NSRS are also being conducted. Data gathered from the above studies suggest that an expanded NSRS is needed. As a result, a series of NSRS summative subscales is being developed.

Publications:

Jaeger A-C, Kirch D, Schnur D. The negative symptom rating scale: internal consistency and correlations with positive symptoms, Psychiat Res, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02257-04 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical and Neuroradiologic Abnormalities in Tardive Dyskinesia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Michael F. Egan, M.D., Medical Staff Fellow, Neuropsychiatry Branch, IRP, NIMH

Dr. Robert Alexander, Medical Staff Fellow, NPB, IRP, NIMH, Dr. Alison Reeve, Medical Staff Fellow, Clinical Brain Disorders Branch, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Clinical Neuropsychiatry

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are replicating the results of a preliminary outpatient study in which alpha-tocopherol was found to significantly improve measures of tardive dyskinesia, a disorder seen in patients who have had long-term neuroleptic treatment. The current study is a double-blind, placebo-controlled inpatient study using higher doses than the first study and for longer periods. The 4 patients enrolled so far show no adverse reactions. We will examine measures of involuntary movements, neuropsychological functioning, psychopathology, and awareness of presence of abnormal movements before and after treatment.

We are studying 4 hypotheses. First, alpha-tocopherol significantly reduces tardive dyskinesia's severity (as measured by the AIMS rating scale), and this change is not due to fluctuations in medication levels. Second, the disorder is associated with abnormalities on MRI scans, including a higher incidence of enlarged ventricles and cortical sulcal prominence compared to age- and gender-matched psychiatric controls. Third, patients with the disorder have lower average IQs than matched controls and do significantly worse on neuropsychological tests of striatal functioning. Finally, alpha-tocopherol improves measures of negative symptoms (as measured on the NSRS) and awareness of movement disorder (as measured by a self-awareness scale).

Project Description:

Objectives: Tardive dyskinesia (TD) is a significant public-health problem affecting 20-40 percent of patients treated chronically with neuroleptics. A recent review of the literature shows no proven, effective treatments. Palliative relief can be obtained by using higher doses of neuroleptics, but this carries the risk of exacerbating the disorder in the long run. A preliminary study done on 15 patients at Saint Elizabeths Hospital (SEH) has shown that alpha-tocopherol may be effective in treating TD's symptoms.

The purpose of this study is to replicate the initial results of the preliminary study using a tightly controlled, double-blind inpatient protocol with live and videotaped sessions rating patients' severity of movement disorder. The need for a tightly controlled inpatient study arises from 2 observations. First, in reviewing the history of research in TD, many compounds initially thought to have significant beneficial effects were later shown to be ineffective. The reasons for this are myriad, but the conclusion is that well-controlled replication is essential. Second, the disorder itself is subject to fluctuation for a variety of reasons, including stress, medication levels, and, most likely, many other poorly studied variables. To control for these as much as possible, an inpatient setting is preferable.

Methods Employed: Patients are recruited and screened from various sources, including SEH's inpatient and outpatient populations, local hospitals and physicians, and traditional NIMH national sources. Those fulfilling the study criteria are admitted for 3 months. Following routine screening to rule out other causes of movement disorder, patients are enrolled in the protocol and receive an MRI scan, neuropsychological testing, and several standardized rating scales. A self-perception scale designed to measure a patient's awareness of involuntary movements is also administered at the beginning and end of the study. Weekly ratings for movement disorder are done using the AIMS scale and videotaped for additional ratings at the study's completion. Psychopathology is followed using the Negative Symptoms Rating Scale (NSRS) to look at negative schizophrenic symptoms and the BPRS. Blood levels of neuroleptics, anticholinergics, and alpha-tocopherol will be monitored intermittently. At the close of the study, patients will again be assessed on several neuropsychological measures. Response to treatment will be looked at taking into consideration MRI data, performance on neuropsychological testing, duration and severity of illness, and numerous demographic factors.

Major Past Findings: In a previous outpatient study, patients demonstrated a significant (43 percent) reduction in AIMS scores after treatment with alpha-tocopherol when compared to placebo. Furthermore, 7 of 15 patients showed a greater than 50 percent reduction in the AIMS. This latter group showed a significant difference when clinical variables were examined in that their total duration of illness was significantly shorter (1.13 vs. 3.29 years).

Significance to Mental Health Research: Tardive dyskinesia affects 20 to 40 percent of psychiatric populations treated with neuroleptics. This iatrogenic illness is at times disfiguring, leading to difficulty in reintegrating the chronically mentally ill into society. Furthermore, TD itself can progress to the point of causing impairment in chewing, swallowing, and breathing, leading to significant morbidity and mortality. Since no good treatment exists, any advance in understanding the pathophysiology of the disorder or its treatment will have

great impact in patient care and future research. A preliminary study has shown that alpha-tocopherol may be effective, implying that free radical formation and resulting neuronal injury may be important in the genesis of the disorder.

Proposed Course of Project: One patient has completed the protocol to date; 3 more are currently enrolled. We anticipate entering 2 patients per month in the study. To complete the project will require a total of 30 patients over the next 18 months.

Publications:

Jeste DV, Wyatt RJ, Weinberger DR, Teychenne PF. Cerebral atrophy in elderly patients: effects of aging and of long-term institutionalization and neuroleptic therapy. In: Miller NE, Cohen JD, eds. Schizophrenia and Aging. New York: Guilford Press, 1987;109-18.

Jeste DV, Iager AC, Wyatt RJ. The biology and experimental treatment of tardive dyskinesia and other related movement disorders. In: Arieti S, Berger P, Brodie HK, eds. American Handbook of Psychiatry 2nd ed. New York: Basic Books, 1986;536-80.

Cadet JL, Lohr JB. Free radicals and the developmental pathobiology of schizophrenic burnout, Integr Psychiatry, 1987;5:40-8.

Lohr JB, Lohr MA, Wasli E, Hilliard B, Larson L, Vardiman E, Wade L, Jeste DV. Self-perception of tardive dyskinesia and neuroleptic-induced parkinsonism: A study of clinical correlates, Psychopharmacol Bull, 1987;23:211-4.

Cadet JL, Lohr JB, Jeste DV. Tardive dyskinesia and schizophrenic burnout: The possible involvement of cytotoxic free radicals. In: Henn FA, DeLisi L, eds. Handbook of Schizophrenia, vol. 2. Amsterdam: Elsevier Science Publishers, 1987; 425-38.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02258-04 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Quantitative Neuropathology of Aging and Neuropsychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

James B. Lohr, M.D., Medical Staff Fellow, NPB, IRP, NIMH

Dr. Dilip Jeste, Department of Psychiatry and Neurosciences, University of California at San Diego; Dr. Joseph Parisi, Chairman, Department of Neuropathology, Armed Forces Institute of Pathology; Dr. Francine Benes, Department of Psychiatry, McLean Research Center

COOPERATING UNITS (if any)

Dept. Psychiat. Neurosci., Univ. of California at San Diego; Dept. Neurol., George Washington Univ. Hosp., Washington, D.C.; Dept. Neuropathol., Armed Forces Inst. Pathol., Bethesda, MD; Dept. Psychiatry, McLean Research Center, Boston, MA

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Aging

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

0.5

OTHER:

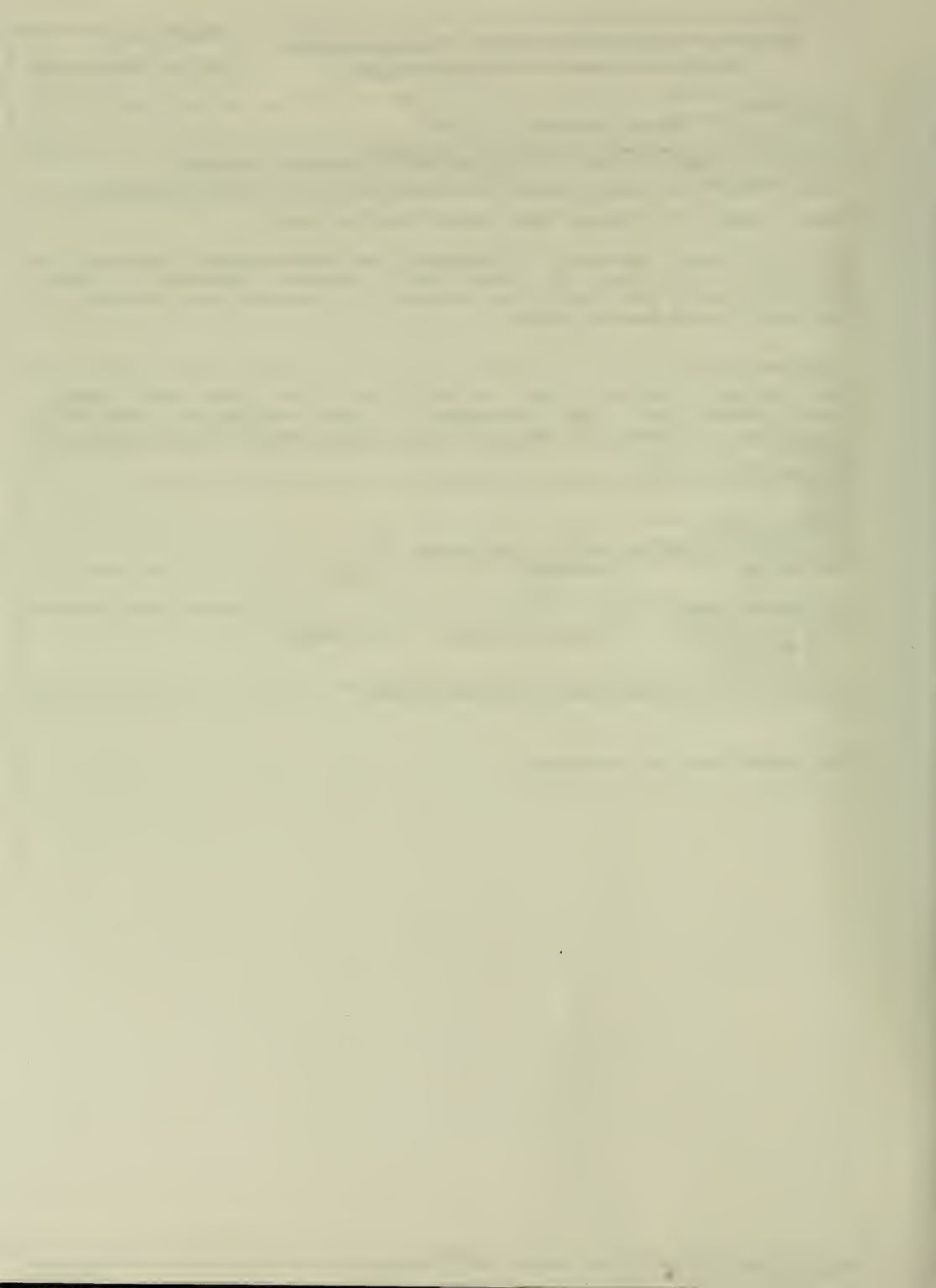
0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02259-04 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Peripheral and Central Catecholamine Turnover in Mental Illnesses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Farouk Karoum, Ph.D., Neurochemist, Neuropsychiatry Branch, IRP, NIMH

Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Psychopharmacology

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

65

PROFESSIONAL:

.65

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Combined gas chromatographic mass spectrometric methods previously developed to assay biogenic amines in various biological media have been employed to assess total body turnover of norepinephrine (sum NE) and dopamine (sum DA) in human subjects and rats. We have also compared changes in these chemicals after a number of pharmacological manipulations in rats. The aim of these animal studies was to gain an insight into how these pharmacological treatments influence brain catecholamines in depression, schizophrenia, and hyperactive children.

(1) Consistent with our 1985 and 1986 Annual Reports, we have continued to gather additional supportive data that suggest a tendency for sum NE to be elevated in major depression. We have also observed a positive correlation between urinary-free cortico and urinary NE and vanilmandelic acid (VMA), a major metabolite of NE in the periphery in humans.

(2) Total body NE and DA turnover were assessed in both hyperactive children and adults after several pharmacological manipulations. The results indicated a correlation between therapeutic benefit and changes in both sum NE and DA irrespective of the direction of change.

(3) The effects of 4 commonly used antidepressant treatments on rat peripheral and central catecholamines were evaluated. A good correlation between the effects of these drugs and sum NE and sum DA in humans and rats was observed. It is suggested that because of this correlation, changes in the rat brain amines observed probably resemble the changes these treatments induce in the human brain. The treatments were chronic zimelidine, desipramine, electroconvulsion, and lithium.

(4) We are currently attempting to reproduce our initial study on DA and NE turnover in schizophrenia and hope to also include patients with tardive dyskinesia.

Project Description:

Objectives: To assess and determine the role of peripheral and central catecholamine in mental illnesses.

Methods Employed: All biochemical analyses were performed by combined gas chromatographic mass spectrometric methods developed in this laboratory.

Major Findings: We have continued to employ the approach described in the 1987 Annual Report: to evaluate the total body turnover of catecholamine in depression and schizophrenia. This approach has been extended also to evaluate total body turnover of catecholamine in the experimental animal following various pharmacological manipulations. We hope to correlate our findings in this latter investigation with the dynamics of the drugs transported from the periphery into the brain and with the profile of the drugs' metabolism.

Significance to Mental Health Research: Our findings, which are related to total body turnover of catecholamines in depression, schizophrenia, and hyperactivity in children, have convinced us that the methods employed are useful in studying the role of catecholamines in mental illnesses.

Proposed Course of Project: Our investigation has been extended to include other antidepressants; we continue to use rats to correlate total body amine turnovers with central amine turnover and metabolism. Our methods accurately and reliably measure a variety of biogenic amines in biological materials so we include, whenever necessary, information on the disposition of such amines as phenylethylamine, tyramine, and the indoleamines. In addition, we are pursuing the correlation of changes in catecholamine turnover in both human subjects and the experimental animal with drug concentrations in plasma, urine, and other biological media.

Emphasis in all future investigations will be directed towards understanding how neuroleptics influence both central and peripheral dopaminergic systems. In these studies we plan to carry out simultaneous measurements of sum DA and sum NE as well as plasma and CSF concentrations of catecholamines and their metabolites in schizophrenics while on and off neuroleptics.

We are currently collecting more urine from schizophrenic patients both on and off medication. We are also collecting urine from patients with tardive dyskinesia.

Publications:

Roy A, Linnoila M, Karoum F, Pickar D. Urinary-free cortisol in depressed patients and controls: Relationship to urinary indices of noradrenergic function, Psychol Med 1988;18:93-8.

Karoum F, Karson CN, Bigelow LB, Lawson WB, Wyatt RJ. Preliminary evidence of reduced combined output of dopamine and its metabolites in chronic schizophrenia, Arch Gen Psychiat 1987;44:604-7.

Roy A, Karoum F, Linnoila M, Pickar D. Thyrotropin-releasing hormone test in unipolar depressed patients and controls: relationship to clinical and biological variables, Acta Psychol Scand, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02262-04 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Electroretinography in Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Myles Jaffe, O.D., Ph.D., Senior Staff Fellow, Neuropsychiatry Branch, IRP, NIMH

Gregory Straw, M.D., Medical Staff Fellow, NPB, IRP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Aging

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Evaluation of the visual system within the context of neuropsychiatry has revealed that psychoactive drugs do affect the retinal function of humans. Metoclopramide, L-dopa, benzodiazepines, and morphine each have an individual effect. In addition, midline dysgenesis in patients with Kallmann syndrome has been investigated. Patients show a higher incidence of congenital color vision loss than expected in the normal population, iris midline defects are present (not previously described), synkinesis was demonstrated in a large percentage of patients, and eye movement disorders have been characterized.

Project Description:

Objectives: To evaluate the actions of a variety of neuropsychiatric drugs on the central nervous system (CNS) of human subjects using an objective bioassay. The tissue we studied was the retina.

Method Employed: Recordings were made using the ganzfeld electroretinogram (ERG), a non-invasive tool that records primarily from the photoreceptors and other cells within the inner neuronal layer of the retina. Three classes of drugs were tested: specific (D-2) dopamine blockers, benzodiazepines, and opiates. Following collection of baseline data, one of the drugs was infused and allowed to equilibrate before a second ERG was obtained.

Major Past Findings: When Parkinsonians had an ERG recorded just after a drug holiday, the waveforms were depressed. When a second ERG was obtained following infusion of L-dopa, the ERG rebounded in the direction of normal. Previous studies showed similar findings using the visual evoked potential but the locus of dopaminergic activity in the visual system could not be localized. Work that preceded the above studies was performed primarily in lower mammals.

New Findings: Metoclopramide, a D-2 blocker, attenuates both rod and cone activity. This phenomenon is more dramatic under conditions of dark-adaptation. Since one of the CNS loci with the highest density of D-2 binding sites is the outer segments of the photoreceptors, our results have shown functional effects of these retinal D-2 receptors.

Diazepam has also been shown to attenuate the function of both the rods and cones in humans; this effect is more pronounced under conditions of light-adaptation. Our findings are linked chronologically close to the new description of benzodiazepine receptors in the human retina. Our results may describe some of the functional properties of the retinal benzodiazepine receptors. In addition, diazepam has been shown to reduce the photomyoclonic reflex in humans. This is an artifact of testing that can be particularly confounding in patients with compromised retinal function. Elimination of this artifact may help in more correct diagnostic procedures and suggests that diazepam could be useful as a provocative agent in retinal testing.

Morphine also affects the electroretinogram although its effect is primarily on the timing mechanism underlying the processing of light flashes. Since the early 1980s, opiate receptors have been known to exist within the retina; we have described some of their functional properties.

Additional studies in patients with midline dysgenesis have shown that their incidence of mild color vision loss, although higher than in the general population, is not diagnostic of their disease. We have also shown that the process of midline dysgenesis that causes hypopituitary-hypogonadism also gives rise to subtle destruction of the iris midline. There are also eye movement disorders associated with dysgenesis of the cerebellar midline and synkinesis, the origin of which is difficult to localize.

Further studies have lead to advancements in the science of bioadhesives. Using an explant tissue culture model, we have devised a method to test the toxicity of bioadhesives currently used in ophthalmic- and neuro-surgery. The model may prove useful in future development of better bioadhesives with applications in spinal cord injuries.

Significance to Mental Health Research: The structure of the pharmacological receptors for dopamine and for benzodiazepines is now thought to be more or less uniform throughout the brain. Our current findings suggest that a noninvasive method now exists to evaluate the function of neuroactive agonists in an accessible region of the human CNS.

Proposed Course of Project: Given the current time constraints, we propose that existing data be evaluated, formalized, and published. Future studies could include the application of mathematical models to refine the predictive value of a bioassay that has considerable promise.

Publications:

Jaffe MJ, Currie J, Schwankhaus J, Sherins RJ. Ophthalmic midline dysgenesis in Kallmann syndrome, *Ophthal Paediat Gen* 1987;8:171-4.

Jaffe MJ, Sherins RJ, de Monasterio FM. Is there color vision loss in Kallmann syndrome? (Abstract) *Color Vis Def* 1987;10:26.

Jaffe MJ, Levinson PD, Zimmlichman R, Coen JC, Karson CN, de Monasterio FM. The effect of metoclopramide on the ganzfeld electroretinogram, *Vis Res* 1987;27:1693-1700.

Jaffe MJ, Hommer D. Attenuating actions of diazepam on the photomyoclonic reflex, *Retina* 1987;7:237-40.

Jaffe MJ, Rittmaster R, Wyatt RJ. Attenuating effects of morphine on the human electroretinogram, *Neurosci Abs* 1987;13:1050.

Jaffe MJ, Hommer D, Caruso RC, de Monasterio RM. Attenuating effects of diazepam on the normal human electroretinogram, *Retina*, in press.

Jaffe MJ, Caruso RC, Koppelman M, von Fricken M, Higgins KE. Chiasmal decompression: neurosensory characteristics of incomplete recovery, *Clin Vis Sci*, in press.

Higgins KE, Jaffe MJ, Caruso RC, de Monasterio FM. Spatial contrast sensitivity: effect of age and psychophysical method, *J Optical Soc Am*, in press.

Jaffe MJ, Sherins RJ, de Monasterio FM. Characteristics of color vision loss in Kallmann syndrome, *Doc Ophthalmol Proc Ser*, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02263-04 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Haloperidol Pharmacodynamics and Clinical Response in Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Darrell G. Kirch, M.D., Senior Staff Fellow, Neuropsychiatry Branch, IRP, NIMH

Dr. Llewellyn B. Bigelow, Senior Scientist, Clinical Brain Disorders Branch, IRP, NIMH; Dr. Gregory M. Straw, Medical Staff Fellow, NPB, IRP, NIMH; Dr. Markku Linnoila, NIAAA; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH; Drs. Greg Gerhardt and Robert Freedman, University of Colorado Health Sciences Center

COOPERATING UNITS (if any)

National Institute on Alcohol Abuse and Alcoholism, NIH; University of Colorado Health Sciences Center, Denver

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Clinical Neuropsychiatry

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

As part of a standardized research sequence in the NIMH Intramural clinical program, patients with schizophrenia are withdrawn from neuroleptic medication and, after clinical relapse, treated with a fixed dose of haloperidol. This in turn allows a variety of studies regarding the pharmacokinetics of haloperidol and the effects of haloperidol and other neuroleptics on central nervous system catecholamines. Another pharmacological issue being examined in these patients is drug-drug interactions (specifically involving haloperidol and several other drugs, including nicotine, caffeine, retinoic acids, and ascorbic acid). In addition, basic science investigations regarding these drugs are being conducted.

Project Description:

Objectives: This project uses a high performance liquid chromatography method to measure haloperidol and its reduced metabolite in serum, red blood cells, and tissue samples. Specific problems being studied include the relationship between serum concentrations and clinical variables, pharmacokinetic phenomena, and drug-drug interactions. Other problems being examined include the effects of haloperidol, nicotine, and caffeine on catecholamines. Research regarding the impact of nicotine and caffeine use on patients with chronic schizophrenia is also being conducted. Lastly, the problem of polydipsia and hyponatremia, a relatively common and potentially lethal clinical syndrome that is seen in patients with schizophrenia and that may be related to drug treatment, is being studied.

Methods Employed: The method of quantification of haloperidol and reduced haloperidol involves liquid chromatography after a liquid-liquid extraction process, as previously reported. Research subjects who come into the clinical research program are placed on coded neuroleptics. They are treated with both active fixed-dose haloperidol (0.4 mg/kg/day) or placebo in double-blind fashion. This allows both pharmacokinetic and steady state samples to be collected for serum haloperidol concentrations. Moreover, haloperidol can be measured while the patients are being treated with other drugs, including retinoic acids and ascorbate and blood samples can be collected for quantification of nicotine, cotinine, and caffeine. In addition, basic science studies are being conducted using the administration of haloperidol and other drugs by either injection or infusion via osmotic mini-pumps in rats.

The problem of polydipsia and hyponatremia is being studied by pharmacological approaches (including treatment with demeclocycline, a peripheral vasopressin antagonist) and magnetic resonance imaging.

Major Past Findings: Previous single cell recordings in rats have shown that reduced haloperidol, a metabolite of haloperidol, appears to be inactive. Moreover, studies of the relationship between serum haloperidol concentration and clinical response indicate that, above a threshold concentration (which appears to be approximately 5 ng/ml), patients have the same degree of clinical response regardless of how high a serum concentration is attained. Thus, our own data have failed to show a "therapeutic window" for haloperidol as has been observed by other investigators.

An attempt was made to replicate the earlier finding regarding the relationship between serum concentrations and clinical response. The World Health Organization (WHO) conducted a multi-site study in which patients were given a fixed high or low dose of haloperidol, serum concentrations were measured, and clinical responses were assessed. Initial analyses of the data regarding clinical response at 4 weeks again showed that above a certain threshold, patients had a comparable response and there was no evidence of a "therapeutic window." Data from more subjects are being examined for the WHO study.

Pharmacokinetic data have been accumulated regarding the response to an acute dose of haloperidol and the washout from haloperidol after its withdrawal. Initial data analyses showed a peak in serum concentration 3 to 5 hours after acute administration, with a significant correlation between this acute response and ultimate steady state concentration. Withdrawal data have shown an initial

half life of less than 24 hours, with a slower later phase of drug elimination. Smoking has been revealed to lower serum haloperidol concentrations.

Assessments of plasma monoamine concentrations in patients before and after withdrawal from neuroleptic have shown an increase in homovanillic acid and 3-methoxy-4-hydroxyphenylglycol in patients after they were withdrawn from neuroleptics. Only the increase in the latter compound was statistically significant.

In the past, research involving nicotine showed that patients diagnosed as schizophrenic are more likely to smoke than chronically hospitalized patients with other diagnoses. A larger epidemiologic survey was then conducted. The finding of increased smoking among patients with schizophrenia compared with other diagnoses was supported. Moreover, smokers who have schizophrenia were more likely to also have tardive dyskinesia than those who do not smoke. Animal research regarding nicotine showed a decrease in dopamine turnover in striatum, frontal cortex, and hypothalamus when rats were treated with nicotine for a period of 3 weeks.

Data regarding the interaction of haloperidol and retinoic acids in clinical studies and animal models have been gathered and have been reported under a separate project title (Z01 MH 02318-03 NPB).

New Findings: Further confirmation of a significant pharmacokinetic interaction between haloperidol and retinoic acids, both in serum and in central nervous system tissue, has been obtained as reported under a separate project title.

A study of the combined effect of haloperidol and ascorbic acid indicated that the latter compound does not affect serum haloperidol concentrations and does not appear to enhance clinical response in patients with chronic schizophrenia.

Further work regarding nicotine and caffeine has revealed that patients with tardive dyskinesia have significantly higher plasma concentrations of caffeine, and a tendency toward higher nicotine concentrations, than patients without dyskinesia. Basic science studies of the combined effect of haloperidol and nicotine on rat brain catecholamines indicate that this combination has a potent effect on decreasing striatal dopamine turnover. Another rat study has shown that caffeine also may decrease dopamine turnover in the brain when administered chronically.

Preliminary results from a trial using demeclocycline in treating polydipsia and hyponatremia in schizophrenic patients have not indicated a significant beneficial effect.

Significance to Mental Health Research: Haloperidol remains one of the most commonly used drugs in treating schizophrenia. The data produced by this laboratory may help provide a more rational strategy for dosages. The studies regarding nicotine and caffeine not only may increase our understanding of why patients with schizophrenia are so prone to use these drugs, but also may reveal information on the mechanisms underlying dependence on these substances in normal individuals. The studies of catecholamines are directed at clarifying the basic central nervous system neurochemistry involved in schizophrenia. The syndrome of polydipsia and hyponatremia is relatively frequent and can be lethal. It is important to understand its pathophysiology to either prevent it or develop effective interventions.

Proposed Course of Project: Pharmacokinetic studies of haloperidol at this point will focus on the ongoing WHO study as described above. Clinical studies of nicotine and caffeine will focus on clarifying the association between use of these agents and tardive dyskinesia. Basic science studies of these drugs will focus on their effects on dopamine turnover and receptors. The study of polydipsia and hyponatremia will involve completion of the demeclocycline protocol and a magnetic resonance imaging study of these patients.

Publications:

Kirch D. Pharmacology and behavior. In: Weiner J, ed. Behavioral Sciences. New York: Wiley Press, 1987;31-47.

Kirch D, Gerhardt G, Shelton R, Freedman R, Wyatt RJ. The effect of chronic nicotine administration on monoamine and monoamine metabolite concentrations in rat brain, Clin Neuropharmacol 1987;10:376-83.

Illowsky B, Kirch D. Polydipsia and hyponatremia in psychiatric patients, Am J Psychiat, 1988;145:675-83.

Kirch D. Laboratory tests in psychiatry. In: Kaplan H, Sadock B, eds. The Comprehensive Textbook of Psychiatry, 5th ed. Baltimore: Williams and Wilkins, in press.

Kirch D, Bigelow L, Korpi E, Wagner R, Zalcman S, Wyatt RJ. Serum haloperidol concentration and clinical response in schizophrenia, Schiz Bull, in press.

Straw G, Bigelow L, Kirch D. Haloperidol and reduced haloperidol concentrations and psychiatric ratings in schizophrenic patients treated with ascorbic acid, J Clin Psychopharmacol, in press.

Kirch D, Alho A-M, Wyatt RJ. Hypothesis: a nicotine-dopamine interaction linking smoking with Parkinson's disease and tardive dyskinesia, J Cell Mol Neurobiol, in press.

Kirch D, Jaskiw G, Linnoila M, Weinberger D, Wyatt RJ. Plasma amine metabolites before and after withdrawal from neuroleptic treatment in chronic schizophrenic inpatients, Psychiat Res, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02274-04 NPB

PERIOD COVERED
October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Exploration of New Methods for Treatment of Intractable Epilepsy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Janice Stevens, M.D., Medical Officer, Neuropsychiatry Branch, IRP, NIMH

Dr. William J. Freed, Chief, Preclinical Neurosciences Section, NPB, IRP, NIMH;
Ingrid Phillips, M.A., Psychologist, Clinical Brain Disorders Branch, IRP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Aging

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

1.25

PROFESSIONAL:

.75

OTHER:

.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To devise more effective methods for treatment of epilepsy intractable by conventional modern medical therapy or surgical intervention, we explored the feasibility of brain grafts of GABAergic brain tissue to specific brain areas in rat models of epilepsy. We worked with two satisfactory experimental epilepsy models in the laboratory: audiogenic seizures in genetically predisposed rats and amygdala kindled rats.

This work, reported in part in September 1987, was completed in the fall and winter of 1987. Nine rats completed the kindling-endopiriform-graft protocol and 14 GEPRs completed the inferior colliculus or ventricular adrenal graft protocol. As previously reported (September 1987 progress report), kindled rats with cortical (n=2) or cerebellar (n=1) grafts to endopiriform areas showed a transient (2-4 weeks) increase in kindled seizure threshold commencing 4-5 weeks after transplant. GEPRs had no change in latency or intensity of seizures following cerebellar grafts to inferior colliculus. We can now report, in addition, that GEPR latency and severity were unaffected by adrenal medulla grafts in lateral ventricle wall bilaterally.

Project Description:

1) Rats with audiogenic seizures (obtained from Dr. Phillip Jobe) were tested for latency and type of seizures for 4 successive weekly intervals after which 9 animals with stable seizure type were implanted with fetal cerebellar tissue from rats of 15-day gestation. The tissue was placed over the inferior colliculus bilaterally after removal of the occipital poles by suction through burrholes. Viable cerebellar grafts grew on top of superior colliculus in one animal but were encapsulated and remained separate from the host brain tissue. There was no change in seizure threshold in the grafted animals. Injury to the inferior colliculus by the suction procedure occurred in 2 animals and was associated with elevation of seizure threshold. A second series of 6 rats was implanted with adrenal tissue in lateral ventricle bilaterally. No change was observed in convulsive threshold or intensity in rats tested 1-8 weeks post transplant. Viable grafts were visualized in ventricle wall but contained few tyrosine hydroxylase-positive cells.

2) Twenty Sprague-Dawley rats were kindled to Stage V seizures, after which they were implanted in endopiriform area bilaterally with 15-day gestation fetal cerebellar or cortical tissue. Three of 9 animals completing these experiments (2 with cortical graft, 1 with cerebellar graft) improved (kindling threshold rose by 2 times or more times) transiently between the 3rd and 7th week post-grafting. All reverted to previous seizure threshold thereafter. Pathological examination indicates that grafts grew well intraparenchymally but were in a majority of cases dorsal to endopiriform area. Cortical grafts were equally successful as cerebellar grafts. Glutamic acid decarboxylase immunohistochemistry, a good marker in our hands for the enzyme in adult cerebellum, was not very successful in staining grafted tissues.

Significance: These are among the earliest trials using brain transplants in the search for a new treatment for epilepsy. Although these experiments have not proven very successful, we have learned a good deal that should improve success with future attempts. Above all, we learned that undivided transplanted cerebellar tissue remains in an encapsulated tumor-like mass when implanted in brain or ventricle and probably would not be a satisfactory source of GABAergic cells for transplant. Cortex with its 59% GABA cells or dissociated Purkinje cells may be more satisfactory.

Significance to Mental Health Research: Epilepsy is one of the most prevalent and most disabling neuropsychiatric problems in the United States, and indeed in the world. Epilepsy affects approximately 0.5 percent of the U.S. population. It is largely young people who are most affected, and 20 to 25 percent of those affected are severely and permanently handicapped despite enlightened use of the most modern therapeutic measures. New approaches to both prevention and treatment are urgently required for this disorder.

Proposed Course of Project: This project has been terminated and its results are in press.

Publications:

Stevens JR. Brief psychoses: do they contribute to the good prognosis and equal prevalence of schizophrenia in developing countries? *Brit J Psych*, 1987;151: 393-6.

Stevens JR. Psychiatric aspects of epilepsy, J Clin Psych, 1988;49:49-57.

Stevens JR. Neuropathological findings in schizophrenia. In: See W, Lee C, eds. Transmitters and Ligands in Psychiatry. London:Livingston and Churchill, in press.

Stevens JR. Symptoms of limbic dysfunction in the acute psychoses of Zimbabwe, Int J Neurology, in press.

Stevens JR, Phillips I, de Beaurepaire R. Gamma vinyl GABA in endopiriform area prevents kindled amygdala seizures, Epilepsia, in press.

Stevens JR, Phillips I, Freed WJ, Poltorak M. Cerebral transplants for seizures: preliminary results, Epilepsia, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02275-04 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Search for Virus in CSF and Post-Mortem Brain of Patients with Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Janice R. Stevens, M.D., Medical Officer, Neuropsychiatry Branch, IRP, NIMH

Drs. David Asher and Joan Schwartz, NINCDS, NIH; Dr. David Jacobowitz, NIMH

COOPERATING UNITS (if any)

National Institute of Neurological and Communicable Diseases and Stroke, NIH; George Washington University Neurology Service, Washington, D.C.; Oregon Health Sciences University, Portland; Good Samaritan Hospital, Portland, Oregon

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Aging

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

1.25

PROFESSIONAL:

1.00

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Epidemiologic, immunologic, and pathologic evidence has suggested that, in a significant number of cases, schizophrenia may be related to infection with or activation of a viral disease of the brain. To further pursue this possibility, we have extended our immunocytochemical and in situ hybridization studies of brain (in which we sought evidence of specific viral antigens or genomes without notable success) to incubation of freshly drawn cerebrospinal fluid (CSF) from patients with schizophrenia and from normal and neurological control subjects with human neuroblastoma cells. Results of these studies suggest cell transformations by an element in CSF of patients with chronic schizophrenia.

Project Description:

Objectives: The purpose of this investigation is to search for evidence for an infectious or toxic agent as a significant etiologic factor in schizophrenia or a subgroup of schizophrenic patients. This work was stimulated by evidence from a number of sources including epidemiologic, immunologic, geographic, and neuropathologic studies compatible with an infectious etiology in this disorder. Highlights of the evidence include increased immunoglobulin in the cerebrospinal fluid of schizophrenic patients to specific viral agents CMV, HSV; seasonal birth peaks of schizophrenic patients; uneven geographic distribution; abnormal response of lymphocytes to specific mitogens; toxic effects of urine, serum, or cerebrospinal fluid of schizophrenic patients on animal behavior and tissue cultures; and gross and histologic neuropathologic changes in the brains of individuals with schizophrenia. Our previous attempts to identify antigens or viral genome from schizophrenic brains or passage of this disorder to animals or through cell cultures have generally been negative. We have now, however, just enough positive results to require extension, replication, and introduction of more sensitive methods for identification of our transmissible factor. We are in an unusually favorable position to make such investigations.

Methods Employed: Two groups of schizophrenic patients and controls have been studied:

1. 13 drug-free patients with chronic schizophrenia from the research wards of NIMH's St. Elizabeths Hospital (SEH) and 15 controls (7 patients with neurologic disorders from George Washington University's neurology service and 8 normal controls from SEH staff); and
2. 11 patients with diagnosis of acute schizophrenia or schizophreniform disorder from Oregon Health Science University's psychiatric admission ward and 13 matched controls from Neurologic and Neurosurgery Services of the Oregon Health Sciences University and Good Samaritan Hospital, Portland, Oregon.

Freshly drawn cells (and supernate for SEH samples) were incubated for 5 days with human neuroblastoma cells (SH-EP) after which cells were passaged at 2-week intervals for 6-12 months.

Major Findings: Our results were that, in the SEH sample no cytotoxic effect was observed but after 1-6 months, SH-EP cultures that had been exposed to fresh CSF from 10/13 schizophrenic patients grew to 25-100% higher density than 12 of 15 control-CSF-treated cultures ($p < .01$). Transformation was further evaluated by testing colony formation in soft agar. Three of 4 Sc-CSF but only 1 of 13 control CSF-treated cultures showed increased size and/or number of colonies. The agent causing growth to increased density and colony formation has continued to be expressed up to 30 passages, can be transmitted by cell-free media passed through a .45 μ m filter, and in 1 ml aliquots diluted 1:100. Enhanced growth was expressed more often in cultures exposed to cells from Sc-CSF compared with those exposed only to Sc-CSF supernate. Neuroblastoma cells exposed to Sc-CSF also make more adenylate cyclase than SH-EP cells treated with control CSF.

Evaluations for reverse transcriptase, oligoclonal bands, lectin surface markers, abnormal protein in 2D gels or electron microscopic evidence for viral particles have all been negative. SH-EP cells incubated with CSF from Oregon controls, acute schizophrenic patients, and untreated cells all grew to a density equivalent to the Sc-CSF-treated cells in the SEH sample.

Significance to Mental Health Research: Schizophrenia is one of the most disabling neuropsychiatric problems in the United States, and indeed in the world. Schizophrenia affects approximately 1 percent of the U.S. population. It is largely young people who are most affected, and 20-25 percent of those affected are severely and permanently handicapped despite use of the most modern therapeutic measures. New approaches to both prevention and treatment are urgently required. The work is difficult and immediate rewards are few. Because of circumstantial evidence for infection as a significant cause of some schizophrenias, we are focusing our efforts on the search for an infectious agent. We are fully aware that this is "long-shot" research with no immediate promise of answers. The cell transformation observed in 11 out of 13 schizophrenic cases studied at SEH and in no controls is very encouraging. We attribute, tentatively, the Oregon results to possible serum differences. This is now under investigation.

Proposed Course of Project: Our immediate plans for this project are to investigate further the cause of the cell transformation seen to date. Future work on this project is focused on identifying the factor(s) responsible for cell density achieved with SEH Sc fluid and DNA extraction of media and on determining why Oregon samples all grew to higher density than the SEH material. We are also studying the effects of intracerebral inoculation of fresh or frozen CSF and media from "infected" and control SH-EP cells on behavior and brain pathology in newborn mice.

Immunocytochemical studies with frozen and fixed schizophrenic brain specimens are being continued in collaboration with Dr. Maciej Poltorak (Z01 MH 02421-01 NPB). We have tested schizophrenic and control fixed brain specimens with the antibody raised against phosphorylated neurofilaments considered to be a specific finding in soma of neurons in Alzheimer's disease. Many of the schizophrenic and control specimens are showing similar staining. We are studying control Sc specimens with a variety of neurotransmitter-specific antibodies by immunocytochemistry.

Publications:

Stevens JR, Waldman I. Hat size in schizophrenia (letter), Arch Gen Psych 1987; 44:673.

Schwartz JP, Stevens JR. Transmissible agent in schizophrenia? Neurology 1988; 38:5-119.

Stevens JR. The search for an anatomic basis of schizophrenia. In: Mueller J, Yingling E, eds. Perspectives in the New Neuropsychiatry. New York: Karger, in press.

Stevens JR. Small heads and schizophrenia, Arch Gen Psych, in press.

Stevens JR. Schizophrenia and multiple sclerosis, Schiz Bull, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02280-04 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Tissue Transplantation in Primates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Richard Jed Wyatt, M.D., Chief, Neuropsychiatry Branch, IRP, NIMH

Dr. William J. Freed, Chief, Preclinical Neurosciences Section, NPB, IRP, NIMH;
Drs. Donald Price, Cheryl Kitt, and Malon DeLong, Johns Hopkins Hospital

COOPERATING UNITS (if any)

Johns Hopkins Hospital, Baltimore, MD.

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

2.5

PROFESSIONAL:

1.0

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To advance the work already performed in our laboratory with rats, adrenal medulla was grafted to the denervated caudate of the rhesus monkey in our continuing research on brain tissue transplantation. Graft survival has improved. In the most successful animal, the behavioral response produced by the graft has lasted one year. An instrument (the brain grafter) that facilitates grafting was developed.

Project Description:

Objectives: The objective of this program is to transfer knowledge gained from brain tissue grafts in the rodent into primates and eventually into humans.

Methods Employed: These studies involve surgical, histological, and histochemical procedures in primates.

Major Findings: Considerable success has been achieved in grafting embryonic substantia nigra and young adult adrenal medulla into rats to decrease rotational behavior produced by unilateral lesions of the substantia nigra.

We feel that even though grafts have been introduced into humans, it is important to establish procedures in animals intermediate between the rat and humans. For example, it is crucial to know if grafts survive and, if so, how well in nonhuman primates. Our first series of rhesus macaque (*Macaca mulatta*) animals, using both adrenal medulla and rhesus embryonic substantia nigra, was, for the most part, unsuccessful. With one exception, grafts were not found.

A second series was slightly more positive: at least some tissue survived transplantation. Eight mature adult male rhesus macaque animals received a unilateral neurotoxic lesion of the substantia nigra (including A10) region. At least 2 months later, each animal received a unilateral implant of either fetal rhesus substantia nigra tissue or tissue from its own adrenal medulla, and at least 2 months after implantation, each animal was killed for catecholamine fluorescence histochemistry. The first 2 animals (A1 and A2) received fetal substantia nigra. The remaining animals (A3 through A7) received host adrenal medulla tissue.

For the embryonic substantia nigra implants, a rhesus monkey embryo (59 and 71 day) was surgically removed ex utero and placed in lactated Ringer's solution. The brain was dissected to obtain substantia nigra tissue in a manner analogous to that for embryonic rat brain. The region of the midbrain that includes substantia nigra tissue was divided into approximately 0.25 mm³ pieces. This tissue was drawn into a 22-gauge needle with an average of 6 pieces of tissue per injection in a volume of approximately 10 to 20 μ l of Ringer's solution.

After removing bone and dura, the injection needle was lowered into the head of the caudate nucleus with stereotaxic coordinates corrected by X-ray determination of skeletal landmarks. The needle was lowered until it was within the caudate. The tissue was then injected and after 3 minutes the needle was withdrawn.

For the 6 remaining animals (A3 through A8), the left adrenal was taken through a posterolateral retroperitoneal approach. A single longitudinal incision was made through the adrenal capsule and cortex under a dissecting microscope. The adrenal cortex was peeled off and cortical fragments trimmed away. The adrenal medulla was divided into pieces of 0.25 mm³. The tissue in Ringer's solution was drawn into a 22-gauge needle with between 5 and 10 pieces of tissue in a volume of approximately 10 to 20 μ l per injection.

In animal A3, stereotaxic placement of the adrenal graft was used as described above for the 2 animals implanted with fetal substantia nigra. Five injections were made into the caudate of animal A3. In the 5 remaining animals (A4 to A8), to provide more secure anatomic placement of grafts, the caudate was directly

observed. With the aid of a surgical microscope, a window was cut through the body of the corpus callosum exposing the left lateral ventricle and caudate. A 22-gauge needle was inserted into the body of the caudate to inject the tissue.

Surviving graft tissue could be identified by the presence of specific catecholamine histofluorescence in the cell bodies of the implants. Neither animal implanted with fetal substantia nigra had any evidence of surviving catecholamine-containing graft tissue. In contrast, the 6 animals implanted with host adrenal medulla had at least some surviving tissue in the parenchyma of the denervated caudate nucleus. The graft tissue itself appeared relatively healthy, although accumulation of macrophages was noted adjacent to or surrounding some of the graft sites. The only damage to host caudate associated with the implantation procedure was scar formation along the needle track.

Most graft sites were deep within the body of the caudate nucleus along the implant tract. Additionally, 2 graft sites were on the edge of the caudate nucleus. At least some parts of most grafts appeared fused with the brain parenchyma, but there was no evidence of caudate reinnervation. All graft sites demonstrated some diffusion of the catecholamine. Most fluorescent cells retained the typical rounded appearance of adrenal chromaffin cells. A minority of cells developed polygonal shapes, and a few cells appeared to develop nerve-like fiber processes, though these remained within the graft itself. A third series of animals has been more successful but results are preliminary.

In addition to grafting tissue directly into the striatum of monkeys, tissue has been grafted into the frontal cortex. The advantage of grafting into the frontal cortex is that the surgery is considerably simpler than grafts into the striatum and allows for developing surgical procedures that do not require lesioning animals and use of complex stereotaxic placement of grafts. Also, in some cases, animals do not need to be sacrificed to determine results. In this series of 6 animals, over 10,000 adrenal chromaffin cells have survived.

An instrument has been built that allows us to carefully insert the grafts into the striatum using a stereotaxic instrument. This has increased graft survival in monkeys and we are applying for a patent.

The instrument's main grafter consists of a series of cannulae designed to minimize tissue trauma and allow easy, precise placement within the brain. The principle involves the insertion of tissue housed in a protected enclosure; when the enclosure is withdrawn, the graft is left in place without additional pressure. The device has an outer guide cannula and 2 inserts. The first is an occluder used for initial penetration only, after which it is removed. The second insert's inner cannula is fitted with a stylet. The amount of space the tissue occupies, and therefore the size of the tissue, is determined by the spacing of the stylet in the cannula. Tissue is inserted into the tip of the inner cannula with the stylet fixed. The inner cannula is lowered and the tissue left in the brain by lifting the inner cannula while the position of its stylet is fixed.

Stereotaxic Instrument: For use in *Macaca mulatta*, the instrument is designed to fit into a modified Kopf stereotaxic instrument (Model 1404, David Kopf Instruments, Tujunga, CA), although modifications can be made for use in other stereotaxic instruments. The stereotaxic frame assembly is equipped with 2 carriers (Kopf model 1460), a carrier on each frame bar.

Brain Grafter Construction: The brain grafter, of stainless steel or another rigid, sterilizable material, consists of 2 cannula assemblies, A and B. Cannula assembly A has an outside guide cannula and a stylet for making initial penetration into the brain. Cannula A is affixed to a 10-mm long cuff or holder that fits over one end of the cannula. The tubing of cannula A extends 84 mm beyond its 10-mm cuff. The tubing has a .228-mm wall with an outer diameter of 1.65 mm and an inner diameter of 1.193 mm. Stylet A extends 95 mm beyond a holding knob. The knob has been trimmed on one side so that it can easily pass up and down as the 2 carriers vertically move past one another. Stylet A is brought to a point extending 1 mm beyond outside cannula A. A bevel on stylet A is tapered to be continuous with a similar bevel outside cannula A, allowing for smooth penetration into the brain.

Cannula assembly B consists of an outer cannula that extends 94 mm beyond its 10-mm cuff. Its inside diameter is .685 mm, the outside diameter is 1.066 mm, and it has a wall thickness of .177 mm. Its tip is beveled to give a cutting edge for punching tissue. Stylet B, with a diameter of .558 mm, extends 105 mm beyond its 10-mm long cuff.

Cannula assembly B fits into a holder assembly H, which consists of a hollow tube with a 1-mm wide viewing slot cut 1.5 cm lengthwise. On the viewing slot's edge are 1-mm marks for determining the distance between the cuffs of stylet B and cannula B. A set screw in H holds stylet B in a permanent mount in the barrel of the cannula assembly holder H. Thumb screw H on the cannula assembly holder H maintains cannula B in a fixed position in relation to stylet B.

Use of Brain Grafter: During surgery, cannula assembly A with stylet A is stereotactically lowered through a burr hole to a position where the graft is to rest. Stylet A is removed. Cannula assembly B and holder assembly H are used to keep the stylet and the cannula at a fixed distance to allow the donor tissue to be punched and taken into the cannula. Using the millimeter markings on the view slot of holder H to determine the amount of tissue to be grafted, thumb screw H is tightened around cannula B. (For example, when the amount of tissue to fill the cannula is determined to be 2 mm, the 2 cuffs of the cannula assembly are placed 2 mm apart as determined by the view slot and markings.) Thumb screw H is tightened and the cannula assembly is used to punch the tissue to be grafted. Following filling of the cannula assembly B with the punched material, cannula assembly holder H is inserted into cannula assembly A. Stereotaxic carrier B is lowered onto assembly B and locked in by tightening its carrier screw. Thumb screw H is loosened, and cannula assembly A raised until contact is made with cuff B. As cannula B is raised, the tissue is dropped from the cannula and deposited in its proper place. Multiple injections can be made into the same track by simply raising cannula assembly A to the appropriate height and repeating the procedure.

Experience has taught us that cannula B's cross sectional sizes are the smallest that we can reliably use to punch adrenal medulla from the monkey. Dimensions of the other cannula and stylets are determined by cannula B. Preliminary data indicate that the device may be superior to other techniques for transplantation of adrenal medulla into the primate striatum. In a number of sites, tens of thousands of cells have survived; in other sites, only a few cells survive. Despite this inconsistency, the grafter gives better maximum survival of adrenal chromaffin cells than other techniques we have used in monkeys. The yield (survival of cells) using this device is also superior to what we have found in

the parenchyma of the rat brain, where about 200 chromaffin cells per animal survive permanently when simply injected with a needle into the striatum.

Behavioral Response: During the last year, several animals have had multiple grafts placed into the right putamen after having MPTP lesions. The initial response of all animals has been to decrease the apomorphine-induced rotations away from the lesion. This rotation indicates the graft's success in that a failure to rotate after apomorphine suggests that dopamine has been replenished by the graft. One animal has had essentially a complete recovery following multiple adrenal grafts to the putamen. This success has continued for a year.

Research: These studies may lead to the development of tissue transplantation as a therapeutic procedure for degenerative diseases and destructive lesions of the brain in the clinic. Also, they may lead to increased knowledge about development and regeneration in the brain in general. Since there is considerable evidence that some schizophrenic patients have altered brain structure (perhaps through degeneration, and degeneration is clearly involved in diseases such as Alzheimer's), learning more about brain plasticity is of primary importance in understanding these illnesses.

Proposed Course of Project: Brain grafting should be seen as both a potential treatment for disorders such as Parkinson's disease as well as a potential for understanding plasticity in general. The course of this project should continue until such time as there is sufficient justification for bringing these techniques on a widespread basis into the clinic. At that time, further refinements and developments will probably be needed to maximize potential benefits to patients. Because work with primates is inherently slow, progress will also be slow. Nevertheless, there do appear to be incremental enhancements of our ability to graft tissue in primates over the last few years. We would expect this progress to continue over the next several years.

Publications:

Wyatt RJ, DeRenzo EG. Deinstitutionalization: "For every complicated problem there is a simple solution and that solution fails" (H.L. Mencken). Stanford University, in press.

Liebowitz MR, Quitkin FM, Stewart JW, McGrath PJ, Harrison W, Karoum F, Wyatt RJ, Levitt M, Rabkin J, Klein DF. Efficacy of L- deprenyl in atypical depression: A preliminary report. Budapest, Hungary: Chinoin Pharmaceutical Inc., in press.

Potkin SG, Bell KM, Wyatt RJ. The relationship between monoamine enzymes and schizophrenia. In: Handbook of Studies in Schizophrenia, III Psychobiology, in press.

Wyatt RJ, Kirch D, DeLisi L. Biochemical studies of schizophrenia. In: Kaplan H, Sadock B, eds. The Comprehensive Textbook of Psychiatry, 5th ed. Baltimore: Williams and Wilkins, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02281-04 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neural Tissue Microchip Interface

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

A. Paul Oliver, Physiologist, Neuropsychiatry Branch, IRP, NIMH

Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH; Dr. Myles Jaffe, Senior Staff Fellow, NPB, IRP, NIMH; Dr. Marty C. Peckerar, Naval Research Laboratory

COOPERATING UNITS (If any)

Microelectronics Processing Facility, Naval Research Laboratory, Washington, D.C.

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Development of a neural prosthesis requires fabrication of a solid state device capable of recording information from neural tissue and stimulating it in a timely manner. We began this process by designing a simpler system to test and monitor cultured neural tissue. We identified some problems (such as suitable insulation for the device and corrosion in a critical part of the system) and we improved the tissue culture system to make the tests as realistic as possible. We have also worked with cultured retinal tissue in the system so that two-way communication could be achieved.

Project Description:

Objectives: Development of practical neural prosthetic devices offers a potential method for replacement of damaged or destroyed neuronal tissue. In the early design stages of this project, many difficult technical problems must be solved. To do this, a simpler system for communicating with animal nervous systems is being developed. One device is part of a tissue culture system for long-term recording of cultured nerve cells; a second device, for *in vivo* application, is being fabricated. Both will be integrated with a computer for signal analysis.

Methods Employed: Both devices are being made with photolithographic techniques at the Naval Research Laboratory. The computer interface electronics have been designed and built by the NIMH Intramural Program Technical Development Service. The tissue culture chip will record a minimum of 30 channels with one or more nerve cells per channel; a Dec Computer will store and analyze the information. The *in vivo* system has 40 channels, and is designed for insertion into brain structures such as cerebral cortex. It will be used for simultaneous recording of nerve cells in a cross-section of a given brain structure.

Major Past Findings: The recording system is workable but needs more insulation to prevent signal attenuation. The system is vulnerable to corrosion when used over long periods of time. It is difficult to coat the probe so that cultured tissue will stick to the probe.

Retinal tissue can be cultured and kept *in vitro* for considerable periods of time. However, we have not yet demonstrated light responses from this tissue. We have developed a new technique using a substance called Matrigel, a commercial product that allows retinal growth approximating that seen with *in vivo* retinal transplants. We expect that this will result in a workable system.

New Findings: The prosthesis work has been temporarily delayed for another priority project. The fabrication of a more advanced system is not yet complete.

Significance to Mental Health Research: The development of prostheses and the method of grafting tissue to the brain both offer potential methods for restoring function to damaged tissue. The work done on prosthesis design, and the tissue culture studies will contribute toward the attainment of these goals.

Proposed Course of Project: Development and testing of communication devices will continue in this laboratory.

Publications:

Oliver AP, Wyatt RJ, Peterson DL, Peckerar MC. Fabricated array microelectronics in a system of cultured nerve cells, Proceedings of the first IEEE conference on synthetic microstructures in Biological Research. Washington, D.C., Naval Research Laboratory, 1988;94.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02282-04 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Neurovirology and Neuroimmunology of Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Darrell G. Kirch, M.D., Senior Staff Fellow, Neuropsychiatry Branch, IRP, NIMH

Dr. Rita Anand, Special Expert, NPB, IRP, NIMH; Dr. Anita Feenstra, Visiting Associate, NPB, IRP, NIMH; Dr. Nicholas M. Papadopoulos, Clinical Chemistry Service, NIH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS (if any)

Clinical Chemistry Service, NIH

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Clinical Neuropsychiatry

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The project on neurovirology and neuroimmunology is focused on obtaining evidence that the pathogenesis of schizophrenia and other neuropsychiatric illnesses may involve either an infectious process by a viral agent and/or an autoimmune reaction involving central nervous system tissue autoantibodies.

Project Description:

Objectives: Despite a vast number of studies showing both structural and functional abnormalities in the central nervous system in schizophrenia, the etiology or etiologies of this disorder are unclear. This project is intended to use newly developed techniques in molecular biology, virology, and immunology to study schizophrenia and other neuropsychiatric illnesses and search for a possible viral and/or autoimmune cause.

Methods Employed: Cerebrospinal fluid (CSF) samples are analyzed using rate nephelometry to measure both albumin and immunoglobulin G (IgG). If these components of CSF and serum are measured, one can estimate endogenous IgG production in the central nervous system.

Plasma samples are analyzed for concentrations of interferon as a marker of immune response. Lymphocytes are harvested from patients and established in tissue culture in Dr. Feenstra's laboratory. The methods used in her laboratory to study these tissue cultures for evidence of a retrovirus infection are described under the project heading Z01 MH 02313-03 NPB. In addition, Dr. Anand, a virologist, heads a laboratory that will study the biological effects of retroviral infections in the central nervous system. Tissue culture techniques using both peripheral lymphocytes and brain tissue will be used in an attempt to isolate viruses. Attempts will also be made to search for evidence of a viral infection using DNA and RNA hybridization methods.

Major Past Findings: In past studies, a subset of patients with chronic schizophrenia was found to have increased central nervous system IgG production. In one patient there was evidence of oligoclonal banding when electrophoresis of CSF was performed.

New Findings: Initial attempts to study lymphocyte cultures for evidence of a retroviral infection have been negative. Recent data regarding plasma interferon indicate an increased frequency of elevated interferon concentrations in patients with schizophrenia, a finding that may, in part, be related to neuroleptic treatment.

Significance to Mental Health Research: Although studies in this area have yet to identify firm evidence of viral infection in schizophrenia, the goals of the project remain important. If a viral infection and/or an autoimmune process are found to be involved in even a subset of schizophrenic patients, this would be an important advance in understanding this disorder's etiology. Moreover, the discovery of more effective treatments (or possibly the prevention) of schizophrenia is dependent on better understanding of schizophrenia's cause.

Proposed Course of Project: The project's ongoing emphasis will be on using a variety of tissue culture methods and DNA/RNA hybridization methods to search for viruses in blood, CSF, and tissue samples from patients with schizophrenia. There will also be further studies of CSF proteins, plasma interferon, and interleukin to look for indirect evidence of viral infection and/or autoimmune response.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02311-03 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.).

Ontogeny of Preprocholecystokinin, Proenkephalin and Tyrosine Hydrolase in Rats

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Anne-Marie Duchemin, M.D., Visiting Associate, Neuropsychiatry Branch, IRP, NIMH

Dr. Thanh Tam Quach, Visiting Associate, LDN, NICHD, NIH; Dr. Michael Iadarola, Neurobiology-Anesthesiology Branch, NIDR, NIH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS (if any)

Laboratory of Developmental Neurobiology, NICHD; Neurobiology-Anesthesiology Branch, NIDR, NIH

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Aging

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

0.3

PROFESSIONAL:

0.3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The project on ontogeny of preprocholecystokinin and proenkephalin has been completed. The same cDNA probes have been used to study the regional distribution of preprocholecystokinin and proenkephalin mRNAs in rodent brain.

The levels of preprocholecystokinin mRNA and cholecystokinin peptide were measured in several regions of rat and mouse brain using RNA blot analysis and radioimmunoassay. Comparison with proenkephalin mRNA levels in the same regions was made.

Project Description:

Objectives: Measurement of the mRNA, which codes for the synthesis of the precursor proteins, provides a key element in understanding neuropeptide function. The present study determined the regional expression of cholecystokinin gene in rat and mouse brain.

Methods Employed: Quantification of preproCCK, and proenkephalin mRNA was made by Northern blot analysis using the corresponding cDNA probes. RNA from several brain regions was prepared by the guanidium-thiocyanate-caesium chloride method. RNA was electrophoresed on formaldehyde-agarose gel and transferred to nitrocellulose paper. RNA blots were hybridized with the ³²P-nick translated cDNA probes. Quantitative analysis of the autoradiographs was performed by densitometric scanning. The levels of the neuropeptide transcripts were normalized to the level of β -actin mRNA.

CCK peptide levels were measured in the same brain regions by radioimmunoassay with an antiserum directed against the c-terminal amide portion of CCK-8.

Major Findings: In mouse and rat brain, high levels of expression of cholecystokinin mRNA were observed in neocortical areas, hippocampus, thalamus, and amygdala. Intermediate levels were observed in periaqueductal grey, hypothalamus, substantia nigra, ventral tegmental area, and olfactory bulbs. Little or no mRNA was detected in caudate nucleus, nucleus accumbens, olfactory tubercle, and cerebellum. In contrast, caudate and olfactory tubercle expressed large amounts of preproenkephalin mRNA.

Despite high levels of CCK peptide, the striatum displayed little or no detectable CCK mRNA in either rat or mouse. Thus the majority of CCK peptide must come from afferent projections to the striatum.

Surprisingly, we found high levels of CCK mRNA in thalamus, whereas immunocytochemical studies did not report CCK-containing neurons in this structure, and that CCK peptide levels measured by RIA were low.

Significance to Mental Health Research: These data extend the mapping studies of CCK-containing neurons in rodent brain to provide an additional perspective on sites on neuronal CCK biosynthesis.

Proposed Course of Project: The data have been analyzed and submitted for publication.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02312-03 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurotrophic Activity in Cerebrospinal Fluid of Schizophrenic Patients

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Anne-Marie Duchemin, M.D., Visiting Associate, Neuropsychiatry Branch, IRP, NIMH

Dr. Thanh Tam Quach, Visiting Associate, LDN, NICHD, NIH; Dr. Charles Kaufmann, Staff Psychiatrist, NPB, IRP, NIMH; Dr. Richard Suddath, Medical Staff Fellow, NPB, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS (if any)

Laboratory of Developmental Neurobiology, NICHD, NIH

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Aging

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In a previous study, we found that cerebrospinal fluid of some schizophrenics was able to support survival of sympathetic neurons in culture. This activity was positively correlated with brain ventricle enlargement.

In an attempt to develop an easier assay for the factor responsible for the neurotrophic activity, CSF was tested for neurite-promoting activity on neuroblastoma cells. No correlation was found between the two assays.

Project Description:

Objectives: To confirm the presence in the cerebrospinal fluid (CSF) of some schizophrenics of neurotrophic factors for neurons in culture.

Methods Employed: Sympathetic ganglia from 12-day-old chicken embryos were dissected and, after trypsin dissociation, the suspended cells were plated in the presence of CSF. After 24 hours' culture, the plates were analyzed for neuron survival. Neuron survival was estimated by counting neurons under a phase microscope after fixation and coloration. We developed other methods to quantify neuronal survival. Addition of radioactive tracers to the cell culture and measure of their incorporation in the cells gave an index of the cell survival. We found a good correlation between incorporation of ^{35}S -methionine or ^3H -uridine and the count of surviving neurons and used these tracers in our assays of neurotrophic activity.

Neuroblastoma cell cultures were developed by Paul Oliver and Richard Suddath, M.D., of the NIMH Neuropsychiatry Branch.

Major Findings: We found that CSF from neurologic or non-schizophrenic patients and from normal controls does not allow the neurons to survive in culture. But CSF from some schizophrenic patients was found to contain a neurotrophic activity for sympathetic neurons. Patients with CSF containing the higher neurotrophic activity appeared to have enlarged ventricles as measured on computed tomography. Neuroleptic treatment does not seem to be responsible for the neurotrophic activity. Characterization of this neurotrophic activity showed that it is destroyed by heat. Dilution curves of CSF from controls and from schizophrenic patients showed no evidence of neurotoxicity in controls and that the neurotrophic activity in schizophrenic CSF is dose-related.

Neurite-promoting activity of CSF on neuroblastoma cells did not correlate with their neurotrophic activity on the 12-day-old chicken embryos' sympathetic ganglion neuron assay. The two assays are known to be sensible to different factors; for instance, NGF—which is very potent as a neuron-survival and neurite-promoting factor in sympathetic cells—is inactive on neuroblastoma cells.

Significance to Mental Health Research: Synthesis of some neurotrophic factors is induced by brain injury. The presence, in CSF of schizophrenics, of such factors and determination if they are or not related to brain lesion needs to be confirmed.

Proposed Course of Project: Since neuroblastoma cells cannot be used as an assay of neurotrophic factors from CSF, sympathetic neurons will be used for the studies to follow. A set of 20 CSF from another group of patients and controls will be tested. If the preliminary results are confirmed, purification of these factors by two-dimensional gel chromatography will be intended.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02313-03 NPB

PERIOD COVERED
October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Retroviral Activity in Lymphocytes of Patients with Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Anita Feenstra, Ph.D., Visiting Associate, Neuropsychiatry Branch, IRP, NIMH

Dr. Darrell G. Kirch, Senior Staff Fellow, NPB, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Molecular Neuropsychiatry

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☒ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Evidence that schizophrenia may involve infection by a virus (or viruses) has been indirect. The evidence includes the phenomenology of schizophrenia (insofar as it may be mimicked by some viral encephalitides), epidemiological factors (including a predominance among patients with late winter/early spring births, a north-south gradient, and occasional clustering of cases), and indirect laboratory evidence (gliosis in some neuropathological studies, spinal fluid protein abnormalities, and abnormalities in cell-mediated immunity).

The discovery of the human retroviruses (HTLV-I, HTLV-II and HIV, now also known to affect the central nervous system) and the development of new techniques in human retrovirology made it possible to investigate the role of this class of viruses in the etiology of schizophrenia.

Cultures of peripheral lymphocytes of patients with chronic schizophrenia were established and tested for the retrovirus-specific enzyme reverse transcriptase.

Project Description:

Objectives: Viral infection has been proposed as a possible etiology of schizophrenia. The discovery of several human retroviruses involved in malignancy, as the HTLV retrovirus family has, renewed our interest in this hypothesis. We chose to culture the lymphocytes of patients with schizophrenia because these cells circulate throughout the body, are in contact with the brain, and can be cultured for up to a month without being transformed. As an initial screening, the lymphocyte cultures are tested for the retrovirus-specific enzyme reverse transcriptase. If positive, the cells are stained with different available antibodies against human retroviruses. The positive cells are co-cultured with cells susceptible to viral infection to enrich for viral particles in an attempt to identify and possibly isolate a virus associated with schizophrenia.

Methods Employed: Lymphocytes are isolated from peripheral blood of patients with schizophrenia and matched controls. The cells are cultured after stimulation with phytohemagglutinin (PHA) in the presence of T-cell growth factor. During a period of 30 days the culture is tested for reverse transcriptase activity.

Major Past Findings: Short-term tissue cultures of peripheral lymphocytes from 17 chronic schizophrenic patients and 10 normal subjects were established. The cells were stimulated with PHA and then grown in the presence of T-cell growth factor. The cultures were tested for the presence of reverse transcriptase. No T-cell-associated reverse transcriptase has been detected in our cultures from either patients or normal controls under these conditions.

New Findings: To investigate the possibility that a virus is present but not activated in peripheral lymphocytes of schizophrenic patients, we treated the cells with different concentrations of 5-azacytidine. Azacytidine, a nucleoside analogue of cytidine, is widely used to reactivate viral genes by reducing the methylation state of the genome. Peripheral lymphocyte cultures of 11 patients and 6 controls were established and treated with 2.5 and/or 5 μ M 5-azacytidine. Reverse transcriptase activity was measured in these cultures and compared to the reverse transcriptase activity of untreated cultures. No significant difference was found between the azacytidine-treated and untreated cultures of the schizophrenic patients or the controls.

Proposed Course of Project: Our studies were limited to peripheral T-lymphocytes. A virus associated with schizophrenia might have another cellular tropism and be located only in the brain. To investigate this possibility, postmortem brain material from patients with schizophrenia will be cocultivated with PHA-activated lymphocytes from peripheral blood.

Publications:

Feenstra A, Kirch DG, Bracha HS, Wyatt RJ. Lack of evidence for a role of T-cell associated retroviruses as an etiology of schizophrenia, Biol Psych, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02317-03 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Peripheral and Central Metabolism of D- and L-dopa in Rats

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Farouk Karoum, Ph.D., Neurochemist, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Psychopharmacology

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

.20

PROFESSIONAL:

.20

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Initial work focused on the effects of administering deuterated L-dopa on non-deuterated catecholamines, then shifted towards comparing central and peripheral metabolism of D-dopa with that of L-dopa. Finding that D- and L-dopa give rise to dopamine by about the same efficiency, we characterized these amino acids biochemically and behaviorally.

We carried out additional experiments that explored the underlying mechanisms responsible for forming dopamine from D-dopa. This work completed and terminated the project. The results were published.

Publications:

Karoum F, Freed WJ, Chuang L-W, Cannon-Spoor E, Wyatt RJ. D-DOPA and L-DOPA similarly elevate brain dopamine and produce turning behavior in rats, Brain Res 1988;440:190-4.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02318-03 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effects of Retinoic Acids on Brain, Behavior, and Drug Interactions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Gregory M. Straw, M.D., Clinical Research Associate, Neuropsychiatry Branch, IRP, NIMH

Dr. Darrell Kirch, Senior Staff Fellow, NPB, IRP, NIMH; Dr. William J. Freed, Chief, Preclinical Neurosciences Section, NPB, IRP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Preclinical Neurosciences

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

1.25

PROFESSIONAL:

0.25

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project investigates the pathophysiology of retinoic acid action in vivo. Rats are the current model, but expansion into mouse and guinea pig models is anticipated. The primary focus of the rat model is the pharmacokinetic assay of the interaction of 13-cis-retinoic acid with neuroleptics. Early results have shown statistically significant changes in the blood levels of haloperidol and one of its metabolites after the concurrent administration of 13-cis-retinoic acid to rats. Early results also suggest possible interactions with dopamine metabolism and opiate levels in the central nervous system.

Project Description:

Objectives: (1) To delineate the pharmacokinetical interaction of retinoic acids and neuroleptics in multiple animal species; (2) To define any direct effects of exogenous and/or endogenous retinoic acids on behavior in animal models. The animal models of greatest interest are those that have already shown some ability to detect changes in dopaminergic function or to detect changes in the threshold for seizures or kindling. Many models are sensitive to or require the use of neuroleptics or related drugs. Therefore, the first objective clearly is to define the nature of any pharmacokinetic interaction of the retinoic acids with the neuroleptics.

Methods Employed: Male Sprague-Dawley rats were used in the initial experiments. They received intraperitoneal 13-cis-retinoic acid and subcutaneous haloperidol in 5 doses over 2 and one-half days. The concentration of haloperidol in the blood was then measured by high pressure liquid chromatography. Mice and rats are tested for locomotor activity habituation and for learning and memory.

Past Findings: In the rat, 13-cis-retinoic acid increases the serum haloperidol concentration as well as the hydroxy-haloperidol concentration. Also, as the retinoic acid dose rises, the ratio of hydroxy-haloperidol metabolite to haloperidol drops significantly. Mice show a direct neuroleptic-like effect of isotretinoin in locomotor habituation testing.

New Findings: Anti-neophobic activity in the rat of all trans-retinoid acid is dependent on the dose, the time from dose, and the animal's age. Similarly, there is a complex time course of retinoid acid's effect on the benzodiazepine-sensitive behavior of Swiss-Webster mice in a light/dark shuttle box. Retinoic acid alters the expression of neuropeptide Y in the rat central nervous system (CNS). Retinoic acid does not appear to alter carbachol-stimulated phosphotydyol inositol accumulation in rat hippocampus slices in vitro. Retinoic acid does not appear to alter the homogenous distribution of haloperidol in the rat CNS.

Significance to Mental Health Research: Retinoic acids are endogenous compounds known to modulate diverse cellular processes. They are also used clinically in treating skin ailments and are the center of focus for some cancer research. It is important to know the nature of their interactions with drugs given concurrently to patients with psychiatric diagnoses. However, it may be even more important to know if they have direct effects in these same diagnostic entities.

Proposed Course of Project: (1) Proceed with the pharmacokinetic studies of interactions between retinoids and other agents; (2) Use animal behavior models, including locomotor activity, stimulant-induced hyperkinesis, toxin-induced movement disorders, neophobic and photophobic fading, locomotor behaviors, and learning and conditioning paradigms; (3) Measure directly the neurochemical effects of retinoids in vivo, in vitro, and post mortem—focusing on neuroactive amines and peptides and the second messengers such as phosphatidylinositol and CAMP.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02373-02 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Effects of Cocaine on Central and Peripheral Catecholamines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Richard L. Suddath, M.D., Medical Staff Fellow, Neuropsychiatry Branch, IRP, NIMH

Dr. Farouk Karoum, Chemist, NPB, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH; Dr. Ana Hitri, Pharmacologist, NPB, IRP, NIMH; Dr. Ariel Y. Deutch, Assistant Professor, Department of Pharmacology and Psychiatry, Yale University

COOPERATING UNITS (if any)

Department of Pharmacology and Psychiatry, Yale University, New Haven, CT

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Clinical Neuropsychiatry

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

That stimulants can be associated with a paranoid psychosis in otherwise normal individuals is frequently offered as evidence for the involvement of the dopamine (DA) system in psychotic illness. One animal paradigm designed to study this phenomenon has been the chronic administration of amphetamines (AMPH), agents known to enhance release and block uptake of DA. In numerous animal studies, infusion of relatively low doses of meth-AMPH has been associated with depletion of DA and its metabolites, a decrease in tyrosine hydroxylase, a decline in DA receptors, and structural changes consistent with neurotoxicity. These changes have been found most frequently in the striatum, olfactory tubercle, and cortex. To further examine this mechanism, we chose to study the biochemical effects of cocaine, a stimulant known to inhibit the uptake of central catecholamines. We chronically administered cocaine (10 mg/kg) or saline twice daily to rats by intraperitoneal injection for 1, 2, and 3 weeks. In a series of experiments, the frontal cortex, nucleus accumbens, caudate nucleus, and hypothalamus were removed at intervals ranging from 1 hour to 3 months after the last dose. Norepinephrine, DA, and their metabolites were measured by mass-fragmentography. We found a persistent, significant reduction in dopamine and in the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in the frontal cortex and hypothalamus, an observation lending further evidence to the links between stimulants and persistent dopamine deficit states.

Project Description:

Objectives: The primary objective of this investigation is to assess the suitability of using rats chronically treated with cocaine as an animal model of schizophrenia. We intend to further characterize the effects of chronic cocaine administration in the rat on the turnover of biogenic amines, on receptor physiology, and on indicators of neuronal toxicity, including immunohistochemical staining and quantitation of neuropeptides. We also intend to study the interactions of other pharmacological and non-pharmacological interventions clinically relevant to schizophrenia and other psychotic illness with this paradigm.

Methods Employed: All biochemical assays of biogenic amines and their metabolites will be performed by combined gas chromatographic mass spectrometry. We also intend to explore the use of combined high pressure liquid chromatography and mass spectrometry (LC-MS) for the identification and quantitation of metabolites. Immunohistochemical studies will be done and will involve staining of tyrosine hydroxylase and dopamine beta hydroxylase-positive fibers in the frontal cortex and striatum. Receptor physiological studies will also be done.

Major Findings:

1. Chronic cocaine administration (10 mg/kg) for periods ranging from 1 to 3 weeks significantly reduced dopamine turnover in the frontal cortex, caudate, septum, and nucleus accumbens during treatment.
2. One week after cessation of cocaine administration, dopamine turnover had normalized in all brain regions measured except the frontal cortex and hypothalamus.
3. To further assess the effects of chronic cocaine on central dopamine metabolism, we administered cocaine 10 mg/kg to rats for 1 week, and measured frontal cortical and hypothalamic dopamine and its metabolites 6 weeks and 3 months after termination of administration. In the frontal cortex, combined molecular concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) remained significantly reduced at 6 weeks and 3 months. In the hypothalamus, DOPAC + HVA was significantly reduced only at the 6-week point.
4. Our results indicate a persistent reduction of dopamine turnover in the frontal cortex and in the hypothalamus following 1 week's administration of cocaine.

Significance to Mental Health: Our findings are consistent with clinical symptoms and neurochemical abnormalities observed in chronic cocaine abusers. Both cocaine abuse and disturbances in central dopamine have been associated with both acute and chronic psychosis, euphoria, anhedonia, mood disturbance, and with deficit states (flatness of affect, loss of motivation, impairment in attention and concentration). Cocaine abuse has also been associated with an increase in serum prolactin, a finding consistent with a dopamine depletion state. The finding of persistent reduction in central and peripheral dopamine metabolites in this model provides a possible biochemical mechanism for the clinical symptoms observed in cocaine abusers. It suggests that subjects withdrawing from cocaine should be followed for evidence of peripheral and central dopamine depletion. It

also suggests possible therapeutic interventions that may reduce the incidence of relapse in these patients. This finding also provides further evidence for the involvement of the dopamine neurotransmitter system in the deficit, or "negative symptom," state frequently observed in patients with chronic schizophrenia.

Proposed Course of Project: We plan to further characterize the effects of chronic cocaine administration on biogenic amine metabolites, including the possible production of neurotoxic metabolites. We also plan to further study the time course of the effects discussed above and the effects of multiple exposures (simulating cocaine binges) on biochemical measures, receptor physiology, and measures of neurotoxicity. We will investigate both pharmacologic agents (e.g., neuroleptics) and non-pharmacologic interventions (stress paradigms) on this model.

We also plan through clinical collaboration to pursue investigations of central and peripheral dopamine depletion states in chronic cocaine abusers.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02374-02 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Trial of Isotretinoin in Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Gregory M. Straw, M.D., Medical Staff Fellow, Neuropsychiatry Branch, IRP, NIMH

Dr. Darrell G. Kirch, Senior Staff Fellow, NPB, IRP, NIMH; Dr. Myles Jaffe, Senior Staff Fellow, NPB, IRP, NIMH; Dr. Barbara Illowsky, Staff Fellow, Clinical Brain Disorders Branch, IRP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Preclinical Neurosciences

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

0.25

PROFESSIONAL:

0.25

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Open clinical trials of isotretinoin in schizophrenic patients on and off of haloperidol have suggested a possibly beneficial synergism between haloperidol and isotretinoin in treating psychotic illness. This has been supported by the results of animal studies of the direct and synergistic (with haloperidol) effects of isotretinoin on animal behavior. Therefore, this project has been initiated to evaluate these potential effects in a placebo-controlled, double-blind, cross-over study. Also, other possible effects, including changes in visual and auditory sensory processes, will be monitored electrophysiologically.

Project Description:

Objectives: To examine the clinical effects of isotretinoin in a schizophrenic population.

Methods: The patients will have been diagnosed according to DSM-III criteria, and will be followed with serial psychiatric and nursing ratings and medical and psychological testing. Visual and auditory evoked potentials will be tested, as well as neuroleptic drug levels.

Major Past Findings: Decreased haloperidol blood levels and decreased psychiatric ratings for psychopathology have been seen in open clinical trials.

New Findings: Animal studies suggest isotretinoin may have direct effects of possible benefit separate from interaction with haloperidol. Results from the first half of the study (8 patients) support continuation, in that significant changes with benefit in terms of behavior, affect, and cognition have been seen. Dose dependency appears complex for the effects on vision (ERG) and psychology (BPRS), with more subjects required to better define it.

Significance to Mental Health Research: The retinoids may comprise a group of compounds with novel ranges of benefit and side effects that could provide alternate treatments for psychoses.

Proposed Course: Clinical trials are to be completed by June 1989.

NOTICE OF INTRAMURAL RESEARCH PROJECT

201 MH 02375-02 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Seasonality of Birth and Hospitalization for Schizophrenic Patients

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Gregory M. Straw, M.D., Medical Staff Fellow, Neuropsychiatry Branch, IRP, NIMH

Guido Zani, Ph.D., Saint Elizabeths Hospital

COOPERATING UNITS (if any)

Information Systems, Saint Elizabeths Hospital

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Preclinical Neurosciences

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

0.25

PROFESSIONAL:

0.25

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Many investigators have reported seasonal variations in the evident birth and admission rates for schizophrenic patients. This project uses computerized hospital records and weather data to elucidate these possible "seasonal" effects.

Project Description:

Objectives: To gain mathematical and statistical description of any evidence of interaction between cyclic environmental changes and the birth and admission rates of schizophrenic patients.

Methods: Comparison of computerized databases of hospital records, weather data, and astronomical cycles.

Major Past Findings: Other authors report some evidence of links between season of birth and admission rates for schizophrenic patients.

New Findings: We found a 5% cyclicity in the rate of patient admissions for schizophrenia to Saint Elizabeths Hospital. We have extended the project to use newly acquired computerized algorithms in analyzing the interactive time series data for weather and admissions by date.

Significance to Mental Health Research: The nature of environmental effects on birth and admission rates may assist in formulating testable theories for underlying pathologic mechanisms.

Proposed Course: Analysis to be completed by December 1988.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02406-01 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Culture of Intact Mammalian Retina

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

A. Paul Oliver, Physiologist, Neuropsychiatry Branch, IRP, NIMH

Dr. Myles J. Jaffe, Senior Staff Fellow, NPB, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH; Dr. M.A. Von Fricken, George Washington University

COOPERATING UNITS (if any)

Department of Ophthalmology, George Washington University, Washington, D.C.

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

0.4

PROFESSIONAL:

0.3

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We have been developing a method for long-term culture of retinal explants taken from rat embryos. The tissue has been used to test for toxic effects of prospective adhesives involved in the repair of torn retinas. The system is also being developed as a transducer in co-culture with colliculus for basic studies in neurophysiology and pharmacology. The tissue can be maintained in a normal state for at least 4 days, enough for the toxicity study. We have tested Matrigel, cyanoacrylate, Celltak, and blood components for toxicity and as a substrate for growth and healing. None of the test materials provided an optimum combination of tensile strength and growth substrate properties.

Project Description:

Objectives: The retina is a well-described, easily accessible neuronal structure in embryonic and neonatal rats. Maintained in vitro, it allows controlled studies not possible in vivo. At present, there is no satisfactory solution to a major problem in retinal surgery: repair of large tears. Adhesives are an alternative to surgery—if they are non-toxic and sufficiently strong. Therefore, culture of retina provides a useful way to assess toxicity. In vitro retina also may be used as a transducer in co-culture with its target structures in brain to provide a structured stimulus for studies in information processing. In particular, when coupled with a multi-channel recording system, temporal and spatial analysis of signal processing may become possible.

Methods Employed: Twenty-day-old rat embryos were used to provide the explants. Retinas were dissected free and cultured with standard technical methods. The adhesives were plated prior to the introduction of explanted retinas. The whole system was maintained in culture medium and placed in a temperature-controlled incubator. Cultures were evaluated by phase contrast microscopy (whole tissue) and by sectioned-stained mounts with conventional microscopy. Observations were made at 1-, 2-, and 7-day intervals. Tissue was evaluated for growth and for preservation of lamination.

Major Past Findings: Retinal tissue can be cultured with no loss of cellular integrity for at least 4 days, and with some cell loss, up to 7 days. Four days allow ample time to test adhesive and growth properties of prospective materials. Not one of the materials used (Matrigel, Celltak, blood product, and cyanoacrylate) was optimum for growth or adhesion. The system is a useful test method.

Significance to Mental Health Research: Systems of this type may be useful for testing materials of interest in neurosurgery and in understanding factors affecting the use of implants.

Proposed Course of Project: Further refinements of technique will be developed to extend the lifetime of the tissue in vitro and to maintain its structural integrity.

Publications: A publication is being prepared for submission to the journal RETINA.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02407-01 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Growth Factors in Human Cerebrospinal Fluid

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

A. Paul Oliver, Physiologist, Neuropsychiatry Branch, IRP, NIMH

Dr. Richard Suddath, Medical Staff Fellow, NPB, IRP, NIMH; Dr. Anne-Marie Duchemin, Visiting Associate, NPB, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We have been testing for the presence of growth factors in the cerebral spinal fluid (CSF) of normal and schizophrenic human subjects. The test system uses neurite growth in cells from a neuroblastoma cell line (Neuro 2-A) to assay the presence of growth factors. Indirect growth factors that cause cells to secrete a diffusible growth substance have been demonstrated. However, the distribution does not discriminate between normal and schizophrenic subjects in specimens tested so far.

Project Description:

Objectives: It is possible that growth substances may be present in the CSF of schizophrenic subjects; therefore, it would be desirable to have a simple, fast assay for detecting their presence. The objective of these experiments is to develop and test a bioassay system that could achieve this goal.

Methods Employed: The CSF came from a bank maintained in NIMH's Saint Elizabeths Hospital facility. The cell system selected was a well-characterized neuroblastoma cell line known to be sensitive to picomole quantities of growth-enhancing substances. The cells, purchased from the American Type Culture Collection, are grown in small flasks and can be readily harvested for the assay. After harvesting, they are washed and counted. Cells are aliquoted into a 96-well plate (5000 cells per well) and inoculated with the CSF and several control substances. After incubation for 4 hours at 37 degrees C, the growth of neurites is analyzed for each specimen. The analysis is accomplished by counting the number of cells with neurites per square millimeter in each test well. Numbers derived from control human subjects, schizophrenic subjects, and control substances are compared to evaluate relative growth numbers.

Major Past Findings: Growth-enhancing substances are present in both schizophrenic and normal CSF. However, these substances may not have a direct effect on the test cells. It is possible that the experimental conditions are causing the cells to respond by secreting a diffusible growth substance that secondarily enhances neurite growth. Whatever the mechanism, it has not been possible to demonstrate a clear-cut difference between schizophrenic and control subjects.

New Findings: We found a method that may exclude the possibility of confusing direct and indirect effects by reducing the numbers of cells per test well.

Significance to Mental Health Research: It could be very helpful in understanding the pathology of schizophrenia to demonstrate and isolate a substance from CSF characteristic of the disorder.

Proposed Course: This work will be continued with the new technique until a definite conclusion can be made about its usefulness in assaying abnormal CSF.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02421-01 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunocytochemistry of Neuropsychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Maciej Poltorak, M.D., Visiting Associate, NPB, IRP, NIMH

Dr. William Freed, Chief, Preclinical Neurosciences Section, NPB, IRP, NIMH; Dr. Janice Stevens, Medical Officer, NPB, IRP, NIMH; Dr. Manuel Casanova, Clinical Brain Disorders Branch, IRP, NIMH

COOPERATING UNITS (If any)

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Preclinical Neurosciences

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

.05

PROFESSIONAL:

.05

OTHER:

5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We have been studying the phosphorylated neurofilament (PNF) epitope expression in rodent and human brains. PNF immunoreactivity is not generally found in neuronal axons, except in some pathological conditions where abnormal accumulation of neurofilaments induces neurofilament phosphorylation in perikarya of neurons. We have found that cell bodies of neurons of the nucleus tractus mesencephalici nervi trigemini react with antibodies against PNF in rodent brain. We have also examined the hippocampal and brainstem neurons in control brains and those of patients with schizophrenia and Alzheimer's disease. The results suggest the widespread occurrence of perikaryonal PNF immunoreactivity in human brains. It is possible that the hippocampal and brainstem cell body neurons are predilection sites for normal hypophosphorylation.

Project Description:

Objectives: Neurofilaments are largely phosphorylated in vivo. It has been suggested that these neurofilaments are present exclusively in axons but not in perikarya of neurons in normal brain. Moreover, in pathological conditions, abnormal accumulation of neurofilaments induces neurofilament phosphorylation in neuronal cell bodies. These findings raised our interest in searching for similar changes in brain pathology of other neurologic disorders, including schizophrenia.

Methods Employed: We first examined PNF immunoreactivity in rodent brain sections. We have also evaluated the influence of different preprocessing and fixation methods on staining patterns. The human material consisted of hippocampus and brainstem from controls (n=12) and specimens with the following diseases: schizophrenia (n=12), Alzheimer's (n=5), subarachnoid hemorrhage, glioblastoma, epilepsy, vascular syphilis, and post-encephalitic Parkinson's. Histocytochemistry was performed using the peroxidase-antiperoxidase method. The sections were stained also with hematoxylin and eosin and Holmes silver impregnation.

Major Findings: Our findings were three.

(1) We found that cytoplasm of neurons of the nucleus tractus mesencephalici nervi trigemini in normal rodents reacts with monoclonal antibodies against PNF. This suggests either that PNFs are localized in the perikarya of some normal neurons or that the antibodies used recognized more than PNFs.

(2) We found that immunoreactivity to PNF was observed in hippocampal pyramidal cells in 6 of the 12 schizophrenic brains and in 9 of the 17 control brains (2 with neurological disorders and 7 with nonneurological diseases). As described by others, the immunoreactivity to PNF was observed in tangles and plaques of the Alzheimer's brains. In schizophrenic and control brains, the positive PNF-stained cell bodies generally did not show neurofibrillary abnormalities in adjacent silver-impregnated sections. These results suggest the widespread occurrence and relative nonspecificity of perikaryonal PNF immunoreactivity in human brain. It is possible that the hippocampal and brainstem neurons are predilection sites for normal perikaryonal phosphorylation in human brain.

(3) We also found that immunoreactivity of monoclonal antibodies against phosphorylated epitopes on neurofilaments is highly dependent upon methods used for tissue preprocessing and fixation. In particular, the preprocessing used for paraffin embedding is required for the observation of putative PNF immunoreactivity predominantly in axons.

Significance to Mental Health Research: Our studies of the presence of phosphorylated neurofilaments in cell bodies and their occurrence in pathological conditions showed that these findings have limited pathognomonic value in human schizophrenia.

Proposed Course of Project: Since disturbances in expression of nonphosphorylated and phosphorylated neurofilament in hypothyroid rats have been shown by others and it has been postulated that ethanol may cause delayed neuronal maturation by decreasing thyroid hormone level, we therefore will also investigate the evolution and distribution of neurofilament in early postnatal development of brain exposed in utero to ethanol.

Using the same animal model, we would like to also determine the influence of ethanol on the expression of the family L₂/HNK-1 of neural-cell adhesion molecules (N-CAM, L1, J1, MAG) and development of glial cells using several different markers for glia (vimentine, M1, and C1).

Publication:

Poltorak M, Freed WJ. Normal neuronal cell bodies of the nucleus tractus mesencephalici nervi trigemini react with antibodies against phosphorylated epitopes on neurofilaments, Exp Neurol 1987;97:735-8.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02418-01 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biologic and Molecular Nature of Putative Neuropathic HIV-1 Isolates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Dr. Rita Anand, Special Expert, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS (if any)

Department of Medical Pathology, University of California, Davis; State University of New York, Stony Brook

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Molecular Neuropsychiatry

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have been studying the biological properties of neuropathic strains of human immunodeficiency virus (HIV-1). This work indicates that some HIV-1 isolates from patients with neurological disorders do not kill T4 lymphocytes, despite the fact that they replicate effectively and were from patients with primarily neuropathic disorders. We have also initiated the molecular characterization of these isolates and their comparison with other HIV-1 (immunopathic isolates). In addition to these studies with neuropathic strains of HIV-1, we are involved in defining the potential antiviral properties of rifabutin against HIV-1.

Project Description:

Objectives: Infection by human immunodeficiency virus (HIV-1) results in a spectrum of clinical manifestations ranging from asymptomatic seroconversion to full-blown acquired immunodeficiency syndrome (AIDS). There is increasing evidence that AIDS is frequently involved with central nervous system (CNS) dysfunctions. Although this disorder usually develops after other complications of AIDS, it often is seen as the first major manifestation of HIV infection.

The mechanism of HIV-mediated induction of neurological disorders in AIDS patients is not known. To determine whether HIVs infecting neurologically affected AIDS patients could be classified in one functional group and to verify the findings of minimal neurohistopathology in many AIDS dementia patients, we isolated and studied biological properties of HIVs from patients with CNS disorders as their primary manifestation of the disease.

Understanding the biological and molecular nature of these isolates would further our knowledge of the virus-mediated neurological disorders in general, and would help in developing antiviral therapies.

Methods Employed: Patients were identified with the help of physicians seeing AIDS patients in New York and Atlanta, Ga. Patients' samples of blood, serum, frozen brain tissue, and cerebrospinal fluid (CSF) were obtained as required. Viruses were isolated by cocultivation of patients' samples with peripheral mononuclear cells (PMNC) of normal human donors. Once isolated, the viruses were identified to be HIV-1 viruses by DNA hybridization using Southern blot method and by immunological cross reactivity in antigen-capture assays.

Virus stocks were prepared by growing viruses in human PMNC. The biological properties of replication and cytopathicity were tested following infection in vitro by reverse transcriptase tests of culture supernatants and by enumeration of T4- and T8-positive cells by FACS-assisted mAb analyses.

One of these isolates (termed HIV-1_{BR}) was molecularly cloned in λ gt10 as two fragments. The smaller fragment 3.5 Kb encompassing envelope, Orf-B and LTR, was recloned in plasmid pBR322 and then subjected to DNA sequencing by the dideoxy-chain termination method.

Major Findings: Our studies are the pioneer findings that have indicated the presence of HIV-1 isolates, in neurologically affected patients, functionally dissimilar than the isolates from immunologically affected patients. The significance of these results is that the neurological disorders in AIDS may be caused by a biologically distinct subgroup of HIV-1. These isolates are noncytotoxic to T4+ lymphocytes, the hallmark of HIV-1 infection, but at the same time they replicate efficiently.

We have also found that in one patient isolates from CSF and peripheral blood have same noncytotoxic property. Therefore, neuropathicity appears to be primarily a function of the infecting virus type and not the tissue source.

Our molecular sequence studies of HIV-1_{BR}, still in progress, have revealed that this virus is an entity distinct from any other known HIV-1 isolates. Preliminary sequence analysis indicates the probable presence of an interesting block of sequences in the 3.5 Kb portion of the 3' half of the genome. Further

analyses of HIV-1_{BR}, comparison, and homology searches will be helpful in determining putative neuropathic sites.

Significance to Mental Health Research: The biological and molecular nature of neuropathic HIV-1 and its role in dementia and psychiatric disorders will definitely yield not only technical approaches to searching for such agents in mental disorders, but will also increase the understanding of the mechanism of virus-mediated CNS dysfunctions. Obviously, detailed knowledge of the causative agents leads to better therapeutic strategies.

Proposed Course of Project: The proposed course of this project is to:
a) understand the cellular tropisms involved in infection with these isolates;
b) generate molecular recombinants to identify genetic sites associated with neuropathy; and c) clone and sequence noncytotoxic isolates in an effort to identify genetic sites of biological activity.

It is also our goal to include, very shortly, the studies on psychiatrically affected HIV-1 positive patients and the search for retrovirus etiology of schizophrenia.

Publications:

Anand R, Moore J, Jaffe H, Feorino P, Weinstein R, Curran J, Srinivasan A. DNA and protein heterogeneity in serial isolates of HIV from a blood donor: indication of change in vivo, *Microbios* 1987;52:191-201.

Srinivasan A, Anand R, York D, Ranganathan P, Feorino P, Schochetman G, Curran J, Kalyanaraman US, Luciw PA, Sanchez-Pescador R. Molecular characterization of human immunodeficiency virus from Zaire: nucleotide sequence analysis identifies conserved and variable domains in the envelope gene, *Gene* 1987;52:71-82.

Anand R, Srinivasan A, Luciw P, Gardener M, Dankekar S. Molecular characterization of a human immunodeficiency virus (HIV-1) associated with neuropathology, *Ann Neurol* 1988;23:562-5.

Anand R. Natural variants of human immunodeficiency virus (HIV) from patients with neurologic disorders do not kill T4+ cells, *Ann Neurol* 1988;23:566-70.

Anand R, Moore J, Curran J, Srinivasan A. Interaction of rifabutin with HIV: inhibition of reverse transcriptase, replication and cytopathicity, *Antimicro Agents and Chemoth* 1988;32:684-8.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02419-01 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Spiperone Binding to Human Peripheral Blood Lymphocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Anita Feenstra, Ph.D., Visiting Associate, Neuropsychiatry Branch, IRP, NIMH

Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Molecular Neuropsychiatry

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Peripheral blood lymphocytes and platelets have been used as model systems for some aspects of the central nervous system. It has been demonstrated that lymphocytes express specific binding sites for neuropeptides. Also, the existence of specific spiperone binding sites was described on these cells. Studies are being conducted to characterize the spiperone binding to human peripheral lymphocytes and related cell lines.

Project Description:

Objectives: Specific spiperone binding sites were described on peripheral human lymphocytes. In addition, two studies showed that the number of sites increased in lymphocytes of schizophrenia patients compared to normal controls. The goal of this study is to characterize this spiperone binding site, followed by the evaluation of its potential as a marker in schizophrenia.

Methods Employed: Patients for the study are recruited from both our own research patient population in the William A. White Division and the inpatient and outpatient services of Saint Elizabeths Hospital. Normal controls come from the community.

Blood samples are obtained by a single venipuncture using a heparinized syringe. The lymphocytes are then isolated using a Ficoll-Paque gradient and used for the determination of [3H]-spiperone binding. To measure this binding, cells are washed 3 times with HEPES (25 mM) buffered Hank's salt solution containing 2 mM EDTA. Binding assays are performed in HEPES-buffered salt solution without EDTA. Cells and various concentrations of [3H]-spiperone (0.04 to 3 nM, diluted in the incubation buffer) are incubated in the presence or absence of 1 μ M(+)butaclamol at 37°C for 60 minutes in 1.5 ml eppendorf tubes. After 4 minutes, the reaction is stopped by high-speed centrifugation (10,000 g) and the cell pellet is washed once with ice-cold incubation buffer. Then the bottom of the tube is cut and counted.

Major Findings: This project was begun in November 1987; the findings will be reported in the next annual report.

Significance to Mental Health Research: Establishing a possible vulnerability marker in schizophrenia is of great importance, for it would substantiate and clarify the genetic component of the disease. It also would give direction to future research aimed at understanding the biological basis and abnormalities associated with the disease. The proposed study will help to achieve this goal by characterizing the spiperone binding on lymphocytes and establishing if the dopamine receptor system is such a vulnerability marker.

Proposed Course of Project: Our first research goal is to confirm and characterize the specific spiperone binding to peripheral lymphocytes. After that, we will determine whether the number of spiperone binding sites is elevated in our schizophrenic patient group.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02420-01 NPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of Chronic Exposure to Cocaine on Metabolism of Catecholamines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Farouk Karoum, Ph.D., Neurochemist, Neuropsychiatry Branch, IRP, NIMH

Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH; Dr. Richard L. Suddath, Medical Staff Fellow, NPB, IRP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Molecular Neuropsychiatry

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:

.65

PROFESSIONAL:

.65

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cocaine is a habit-forming stimulant that shares with other drugs of abuse the ability to produce rewarding or "reinforcing" effects. Because of the alarming rise in cocaine abuse during the last 15 years, there is a keen interest in cocaine from both public health and basic science perspectives. Various behavior studies imply a major role of central dopamine in the expression of the pharmacological effects of cocaine, but direct biochemical support for this view is at best very sparse and inconsistent. The latter may be attributed to two important factors. First, most reports on the biochemical effects of cocaine were obtained from acute studies. Second, in the majority of these studies, the striatum and nucleus accumbens were the brain regions frequently analyzed. Therefore, certain important brain regions may have been overlooked.

To correct for the above experimental flaws, we have systematically evaluated the effects of 1 to 3 weeks' chronic cocaine administration on central and peripheral biogenic amines, especially the catecholamines. The results of our investigation revealed a preferential, long-term reduction of dopamine turnover and/or metabolism in both the periphery and the brain. In the brain, the frontal cortex and the hypothalamus apparently are the areas most affected.

Project Description:

Objectives: The primary objective is to assess the suitability of using rats chronically treated with cocaine as animal models of schizophrenia. To accomplish this, the behavioral and biochemical effects of cocaine must be characterized, and the results compared with the various clinical manifestations of schizophrenia. An immediate objective is to first evaluate the effects of chronic cocaine exposure on central and peripheral biogenic amines, especially catecholamines, serotonin, and other neurotransmitters. Once we have successfully completed this investigation, our next objective will be to assess the kinetics of cocaine metabolites and their transport from the periphery into the brain and to determine how certain antipsychotic drugs interact with cocaine—both behaviorally and biochemically.

Methods Employed: All biochemical assays of biogenic amines and their metabolites will be performed by combined gas chromatography mass spectrometry. These methods were developed in this laboratory and have been extensively applied to a wide range of clinical and basic science projects.

To fulfill most of our future objectives, new methodologies will need to be explored. This will involve the use of combined high pressure liquid chromatography and mass spectrometry (LC-MS) as well as tandem mass spectrometry (MS-MS) for the identification and quantitation of metabolic products of drugs. Since MS-MS is a technique that merits exploration in the distant future, we plan to concentrate most of our near-term research effort on exploring LC-MS techniques.

LC-MS is a novel analytical tool that has not been fully explored in neuropharmacology. It is ideally suited to investigations aimed at monitoring the bioavailability of drugs. Further, due to the rapid advances in high pressure liquid chromatography (HPLC) in recent years, it is now possible to custom design HPLC columns that offer separation powers and efficiencies that far exceed the performance of gas chromatography. LC-MS is expected to be extensively employed in the rapid and accurate measurements of drugs in various biological media. Another important application of LC-MS will undoubtedly be in the screening for the presence of drugs of abuse in urine and blood.

Major Findings: We have evaluated the effects of 1-, 2-, and 3-weeks' repeated-daily administration of cocaine on rats' disposition of central and peripheral biogenic amines. Peripheral biogenic amines were assessed by following their rate of excretion. In the brain, catecholamines and their metabolites were measured in the various brain regions—the hypothalamus, frontal cortex, septum, nucleus accumbens, striatum, and hippocampus—that are expected to play some role in the expression of cocaine's central effects.

Chronic cocaine exposure produced a preferential, long-term reduction in dopamine and phenylethylamine turnover and/or metabolism. This effect was detected 6 weeks after termination of 7 days' exposure to cocaine. This result suggests a long-term down regulation of peripheral dopamine turnover, a phenomenon similar to that observed in chronic schizophrenia (Arch Gen Psych 1987;44:604-7).

While acute cocaine treatment (10 mg/kg) was ineffective, chronic exposure to cocaine for periods ranging from 1 to 3 weeks significantly reduced dopamine turnover in the frontal cortex, striatum, septum, and nucleus accumbens during

treatment. One week after termination of treatment, dopamine turnover, as assessed from its rate of metabolism, was normal in all regions analyzed except the frontal cortex and hypothalamus.

To assess the long-term effects of cocaine exposure on central dopamine, we exposed rats to one week of cocaine treatment and measured hypothalamic and frontal cortex dopamine and its metabolites 6 weeks and 3 months after termination of cocaine treatment. The combined molecular concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) remained significantly low in the frontal cortex at 6 weeks and at 3 months. In the hypothalamus, DOPAC + HVA was only low at the 6-week point.

The results of our studies so far suggest a long-term effect of cocaine exposure on dopamine turnover in the hypothalamus and the frontal cortex. Some of the mental impairments frequently observed in cocaine abusers can be attributed to disturbances in frontal cortex and hypothalamic functions. These include cognition, reward, emotional instability, and disturbance in hormone secretions that are regulated by the hypothalamic-pituitary-adrenal axis.

Significance to Mental Health Research: The successful development of an animal model of schizophrenia in itself will be of great scientific value. It will allow the convenient testing of new antipsychotic drugs and will offer better insight into the role of various neurotransmitters in schizophrenia.

Getting to our final goal—development of an animal model of schizophrenia—will be accompanied by new understanding of cocaine's pharmacology and its effects on central amines. These observations will be valuable in aiding future strategies in the treatment of cocaine addiction.

Proposed Course of the Project: We plan to pursue the fulfillment of the near- and distant-future objectives outlined above.

Publications:

Karoum F, Fawcett RW, Wyatt RJ. Chronic cocaine effects on peripheral biogenic amines: a long-term reduction in peripheral dopamine and phenylethylamine production, Eur J Pharmacol, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02428-01 NPB
PERIOD COVERED October 1, 1987 through September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biological Patterns of Intraventricular Grafts		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Maciej Poltorak, M.D., Visiting Associate, Neuropsychiatry Branch, IRP, NIMH Dr. William Freed, Chief, Preclinical Neurosciences Section, NPB, IRP, NIMH; Dr. Nancy Sternberger, Department of Anatomy, University of Maryland; Dr. Ludwig Sternberger, Department of Neurology, University of Maryland; Dr. Melitta Schachner, Head, Department of Neurobiology, University of Heidelberg, FRG.		
COOPERATING UNITS (if any) Departments of Anatomy and Neurology, University of Maryland, Baltimore; Department of Neurobiology, University of Heidelberg, FRG.		
LAB/BRANCH Neuropsychiatry Branch		
SECTION Preclinical Neurosciences		
INSTITUTE AND LOCATION NIMH, Saint Elizabeths Hospital, Washington, D.C.		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We have been studying selected <u>immunohistological patterns</u> of intraventricular cerebellar allografts in host brains. It has been unclear why cerebellar grafts develop relatively normally without external inputs. We have attempted to determine what factors are essential for completion of their development and survival in host brains after implantation. Results indicate that the expression of <u>cell adhesion molecules</u> in transplanted cerebella is relatively undisturbed. Therefore, it is likely that the molecular mechanisms of <u>granule cell migration</u> are operant in grafts, independently of the normal inputs. Moreover, the data suggest that non-radially oriented <u>glial-fibrillary acid protein—positive glia</u>—may also be a substrate for migrating granule neurons in transplanted cerebella. Other results indicate that genetically normal cerebellar grafts can survive in a <u>mutant host environment</u> defective in cell-cell adhesion molecule and myelination. We have found that <u>intraventricular grafts</u>, as compared to <u>intraparenchymal grafts</u>, are bigger and do not produce large gliosis and demyelination of the host brain. The former, however, is not as well integrated with host brains in terms of presence of interconnecting neurites. The internal organization of both intraventricular and intraparenchymal grafts was relatively normal. We have also found that <u>normal phosphorylation</u> of neurofilaments occurs in cerebellar grafts in host tissue. Our results further suggest that, despite the rather similar enhancement of Ia immunoreactivity in host parenchyma (probably on microglia) after allo- and xenografting, only in <u>xenografts</u> does an intense host reaction occur that is capable of destroying transplanted tissue. </p>		

Project Description:

Objectives: Intracerebral grafting has already been used in humans. It has become important to clearly determine the proprieties and possibilities of intracerebral grafts. The cerebellum is a useful tissue for studies of nervous system development since it contains a limited number of cell populations composed in a stable trilaminar organization with very restricted connections. It has been demonstrated that cerebellar grafts develop relatively normally in host tissue despite the absence of external inputs. These studies were designed to determine which factors are essential for completed cerebellar graft development and to demonstrate the immunopatterns of several antigens involved in histogenesis of cerebellar tissue after transplantations. We also wanted to shed some light on immunological features occurring in host brains after allo- and xenotransplantations.

Methods Employed: The intracerebral transplantations were performed on 66 mice and rats. The embryonic donor tissue was stereotactically implanted into lateral ventricle or into brain parenchyma. The procedures were adapted from previously employed techniques. After transplantations, the animal brains were sectioned and stained with hematoxylin and eosin or cresyl violet. Selected sections were stained immunocytochemically with primary antibodies against phosphorylated and nonphosphorylated neurofilament epitopes, myelin associated glycoprotein, myelin basic protein, glial fibrillary acid protein, neural cell adhesion molecule, L1 molecule, J1 molecule, L₂/HNC-1 epitope, Ia antigen, and helper T cell determinants. We have used the peroxidase- antiperoxidase method and indirect immunofluorescence.

Major New Findings: (1) We studied genetically normal cerebellar grafts transplanted into a mutant host environment defective in both cell-cell adhesion molecule and myelination (Quaking mice). The grafts survived and showed generally normal cytoarchitecture with characteristic expression and distribution of neurofilaments. The normal phosphorylation of neurofilaments occurred in grafts. Myelination was normal as well. These grafts formed internal circuits that partially substituted for the absence of the normal complement of afferent inputs. The data support the notion that neurogenesis with alternative connections can occur in transplants.

(2) We have compared the development of cerebellar allografts using intraparenchymal and intraventricular transplantation techniques. Although intraparenchymal grafts were smaller than intraventricular cerebellar grafts, they were better integrated with host brain in terms of presence of interconnecting neurites despite a large glial scar and demyelination of the host brain. The appearance and distribution of phosphorylated and non-phosphorylated neurofilament epitopes resembled those seen in normal postnatal cerebellar development. Abnormal phosphorylation was not observed. Moreover, the organization of synapse-associated antigens was similar. Thus, internal organization of both types of grafts was relatively normal.

(3) We have studied the expression of cell adhesion molecules in mouse cerebellar grafts. We found no significant differences between the development of transplanted cerebellar tissue and the normal (in situ) development of immunostaining patterns of cell adhesion molecules (N-CAM, L1, J1, MAG). Since the trilaminar cytoarchitecture of transplanted cerebellum was maintained, it is likely that the molecular mechanisms of granule cell migration in normal cerebellum are operant in transplanted tissue. Moreover, the expression of L₂/HNC-1 epitope suggests that this carbohydrate epitope is differentially

expressed in the graft situation when compared to in situ grafts. All data indicate that the developmental appearance of cell adhesion molecules occurs independently of the normal complement of afferent inputs, and is consistent with the possibility that the migration of cells from the external to the internal granular layer involves multiple cellular interactions and is present in transplanted tissue.

(4) We have also studied the expression of glial fibrillary acid protein (GFA) in intraventricular mouse cerebellar grafts. We were unable to find the characteristic GFA-positive elongated radial processes of Bergmann glia in the grafted tissue. Since normal granule cell migration was observed in grafts, it is possible that non-radially oriented GFA-positive glia may also be a substrate for migrating granule neurons in transplanted tissue.

(5) We have studied the histochemical features of the immunological reactions to intraventricular allografts and xenografts. Xenografts provoked an intense immunological reaction involving Ia immunoreactive cells and helper and T cells. The results suggest that the process of implantation of tissue and its associated brain injury induces enhanced Ia immunoreactivity in brain parenchyma (most probably on microglia) both surrounding and within both allo- and xenografts. Despite this predisposition to immunological reactions, only in xenografts did the reaction proceed through all of the steps required for a graft rejection response and ultimately lead to destruction of the grafts.

Significance to Mental Health Research: Intracerebral grafting is a rapidly expanding area of neuroscience. It has already been performed on humans for successful treatment of Parkinson's disease. Although today's application of neural grafting is considered for disorders with one known cell type deficit, it is possible that techniques related to neural grafting can eventually be applied to other disorders. This new approach requires further basic studies to determine the proprieties and possibilities of grafting.

Proposed Course of Project: Since grafted cerebellar tissue showed some disturbance in GFA-immunoreactivity, we have planned to study grafted cerebella using several different markers for glial cells (vimentine, M1, C1). Xenografts were formed to induce strong immunological reactions within host brain and host tissue, and showed enhanced Ia immunoreactivity. Ia positive cells are responsible for initiation of immunological reaction. We would therefore like to study the effect of blocking Ia antigens within host brain on the survival of intracerebral xenografts. We also plan to continue immunocytochemical studies using antibodies against cell-adhesion molecules and neurofilaments and apply these methods to intracerebral adrenal medulla grafts to examine the differentiation of adrenal chromaffin cells following intracerebral transplantation. Ultimately these methods will be applied to the examination of defined cell lines following intracerebral implantation, including tumor cells submitted to differentiating factors and genetically engineered cell lines.

Publications:

Poltorak M, Sternberger LA, Freed WJ, Sternberger NH. Neurofilament expression in cerebellar transplants, J Neuropathol Exp Neurol 1987;46:352.

Poltorak M, Sternberger LA, Freed WJ, Sternberger NH. Neurofilament expression in cerebellar transplants, J. Neurochem 1987;485:166.

Poltorak M, Freed WJ. Cerebellar allografts in brain of quaking mice, Exp. Brain Res, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01532-11 LPP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Catecholamine Receptor

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Christopher Hough, Ph.D., Chemist, De-Maw Chuang, Group Chief, LPP-NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Group on Receptor Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:

1.4

PROFESSIONAL:

1.4

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We have previously shown that desensitization of β -adrenergic receptor (BAR) in a model system of frog erythrocytes is associated with internalization of BAR, resulting in down-regulation of the receptor binding sites. Internalized BAR sites are sequestered in endocytotic vesicles which can be revealed by immunohistochemical staining using BAR-specific antibodies. The internalized BARs can be recycled to plasma membrane during resensitization that occurs after removal of the stimulating BAR agonist isoproterenol. We are addressing the question of whether desensitization and internalization involve a change in the transcription rate of BAR mRNA. For this study, we used the BAR present in C₆-glioma cells which express both β_1 - and β_2 -AR with a fast turnover rate. Preliminary results using β_2 -AR selective cDNA show that, indeed, β_2 AR mRNA levels decrease rapidly in response to stimulation with isoproterenol.

The modulation of BAR in the CNS is also under investigation. We have found BARs in clathrin-coated vesicles isolated from bovine brain, suggesting that these receptors are internalized in vivo. Down-regulation of BAR by chronic antidepressant has been implicated in the therapeutic effect of this drug. We have therefore initiated a study to investigate the effect of chronic administration of the tricyclic antidepressant imipramine on the level of BAR mRNA in order to shed light on molecular mechanisms underlying the regulation of gene expression elicited by antidepressant-mediated changes of synaptic activity.

Our previous studies using the system of frog erythrocytes have provided the first evidence suggesting that down-regulation of β -adrenergic receptor (BAR) induced by prolonged stimulation with the BAR agonist isoproterenol is associated with internalization of BAR sites. This receptor internalization is closely associated with the desensitization of adenylate cyclase to BAR stimulation. Internalized BAR sites are sequestered in endocytotic vesicles with molecular weight more than 20×10^6 daltons and are labeled by lipophilic but not hydrophilic receptor ligands. This receptor internalization has also been substantiated by immunohistochemical staining of erythrocyte BAR using antibody raised against guinea pig lung BAR. In untreated cells, most of the BAR staining was found to be located on the plasma membrane. Treatment with isoproterenol resulted in a time dependent increase in BAR staining which appeared to be mainly located in the cytoplasm. The internalized BAR has also been shown to recycle to the plasma membrane during resensitization that occurs after removal of the stimulating agonist.

In an attempt to further explore molecular details involved in the desensitization process, we have addressed the question of whether desensitization and internalization of BAR involve a change in the transcription rate of BAR mRNA. For these studies, we have chosen the system of C_6 -glioma cell line which contains both β_1 and β_2 adrenergic receptors with fast turnover rates. Moreover, both types of BARs in C_6 -glioma cells have been shown to be rapidly desensitized by preexposure to BAR agonists. Our preliminary results using β_2 -AR selective cDNA show that, indeed, in C_6 -glioma cells, β_2 -AR mRNA levels decreased rapidly in response to stimulation with the agonist isoproterenol. Currently, we are also assessing the turnover rate of β_1 AR mRNA during agonist-induced desensitization in an attempt to elucidate whether β_1 - and β_2 -AR mRNA can be differentially regulated. Conversely, we will study whether the turnover rate of BAR mRNA is restored during the process of resensitization. Undoubtedly, these studies will lead to a better understanding of how receptor stimulation controls expression of the receptor protein.

BAR in the CNS also undergoes adaptation. We have found BARs in clathrin-coated vesicles isolated from bovine brain, suggesting that these receptors are internalized in vivo, since transport of proteins into cells involves clathrin-coated vesicles (Chuang et al. J. Neuroscience 6:2578, 1986). Moreover, chronic treatment of experimental animals with antidepressants is known to decrease the number of BAR, thereby causing BAR desensitization (for review, see Chuang and Costa, In "Handbook of Neurochem. Vol 6, pp 307, 1984). This drug-induced BAR desensitization in the CNS has been suggested to be intimately coupled to its therapeutic effect in the treatment of depression. We have also initiated a study to investigate the effect of chronic administration of a tricyclic antidepressant imipramine on the level of BAR mRNA in order to shed light on molecular mechanisms underlying the regulation of gene expression elicited by antidepressant-mediated changes of synaptic activity. In addition, we will study whether lesions of noradrenergic and serotonergic nerve endings alter the transcription of BAR mRNA. Our ultimate goal is to elucidate transynaptic

PROJECT DESCRIPTION:

Z01 MH 01532-11 LPP

factors involved in turning -on and -off the expression of BAR in the CNS and to relate this gene regulation to the action of psychoactive drugs and some forms of mental illnesses.

PUBLICATIONS:

Alho, H., Dillon-Carter, O., Moxham, C.P., Malbon, C.C. and Chuang, D.-M. Immunohistochemical evidence for the internalization of beta-adrenergic receptors in desensitized frog erythrocytes. Life Sci. 42:321-328, 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01559-07 LPP
PERIOD COVERED October 1, 1987 through September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Phe-Met-Arg-Phe-NH ₂ Like Peptides in the Brain and Spinal Cord: Function and Distribution		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) H.-Y.T. Yang, Ph.D., Section Chief, F.A. Majane, Chemist LPP-NTMH		
COOPERATING UNITS (if any) 1) Pertti Panula, M.D., Dept. of Anatomy, Univ. of Helsinki, Helsinki, Finland 2) Khen Jhamandas, Ph.D., Dept. of Pharmacology, Queen's University, Kingston, Ontario, Canada		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Neuropeptides		
INSTITUTE AND LOCATION NTMH, ADAMHA, NTH, St. Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 2.0	PROFESSIONAL: 0.5	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The <u>octapeptide</u>, Phe-Leu-Phe-Gln-Arg-Phe-NH₂ (F-8-F-NH₂) was first detected by the antiserum raised against the <u>molluscan cardioexcitatory peptide</u>, Phe-Met-Arg-Phe-NH₂, and subsequently isolated from bovine brain. F-8-F-NH₂ injected intraventricularly can decrease the analgesic effect of Morphine. F-8-F-NH₂ was found to be highly localized in substantia gelatinosa of dorsal spinal cords. In the study using <u>in vitro</u> and <u>in vivo</u> superfusion of spinal cords, we found that F-8-F-NH₂ can be released by depolarizing concentration of KCl (in vitro) and also by substance P, the sensory transmitter (in vivo). This result further support our hypothesis that F-8-F-NH₂ may have a modulatory role in <u>antinociception</u>. In rats, F-8-F-NH₂ was found to be also highly concentrated in posterior lobes of pituitary glands. In searching for the role of this peptide in the pituitary, F-8-F-NH₂ was found to be below the limit of detection in pituitary glands of Brattleboro rats, diabetes insipidus rats. The result indicates that Brattleboro rat may be an useful model to explore the function of pituitary F-8-F-NH₂. Using Immunohistochemical technique, two strongly F-8-F-NH₂ <u>immunoreactive neuronal groups</u> in rat brains were revealed. One of them was located in the periventricular hypothalamic area and another prominent cell group was found in the nucleus tratus solitarii. Whether these cell bodies project to pituitaries and spinal cords are under current investigation.</p> <p>The proposed course of this study is to investigate 1) function of pituitary F-8-F-NH₂ using Brattleboro rats, 2) release of F-8-F-NH₂ from the spinal cord and the effect of morphine on this release and 3) sources of F-8-F-NH₂ immunoreactive nerve terminals in spinal cords and pituitaries using lesion techniques.</p>		

In our earlier work, a neuropeptide with morphine modulating activity was isolated from bovine brain. This peptide, Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH₂ (F-8-F-NH₂), was found to be highly concentrated in dorsal spinal cord of bovine and rat. Immunohistochemically, F-8-F-NH₂ immunoreactivity was found in nerve terminals of the superficial laminae of the posterior horn. F-8-F-NH₂ immunoreactivity was also detected in spinal cords of other species including human, mouse and guinea pig. however, characterization of these F-8-F-NH₂ immunoreactivity indicated that there is an interspecies molecular heterogeneity.

In order to further explore the physiological role of F-8-F-NH₂ in the spinal cord, release of F-8-F-NH₂ was investigated using an *in vivo* spinal cord superfusion technique. The *in vivo* superfusion experiment was carried out according to Yaksh et al (Brain Research 26:119, 1983) and F-8-F-NH₂ immunoreactivity released into the superfusate was determined by the radioimmunoassay and characterized by HPLC-coupled with the radioimmunoassay. Superfusion of the spinal cord subarachnoid space with substance P (10⁻⁶ M) caused a release of F-8-F-NH₂ immunoreactivity. This F-8-F-NH₂ immunoreactivity released was found to be identical to F-8-F-NH₂ immunoreactive peptide in the rat spinal cord by the HPLC study. The result of this study further supports our hypothesis that F-8-F-NH₂ may have a role in antinociception. In order to facilitate this study, the release of F-8-F-NH₂ was tested in an *in vitro* superfusion system using isolated rat spinal cord. Fresh whole spinal cords were removed from decapitated rats and continuously perfused with Kreb's solution at 37°C, the perfusates were radioimmunoassayed for F-8-F-NH₂ immunoreactivity. Depolarizing concentration of KCl (56 mm) was found to cause a release of F-8-F-NH₂ immunoreactivity. The result further confirms that F-8-F-NH₂ immunoreactivity stored in nerve terminals in the dorsal spinal cord can be released.

In searching for other appropriate target for further exploration of the physiological role of F-8-F-NH₂, detail distribution of F-8-F-NH₂ immunoreactivity in rat CNS and pituitary was studied. Very high concentration of F-8-F-NH₂ immunoreactivity was detected in pituitary glands and furthermore the immunoreactivity was present exclusively in the posterior lobe. We are currently studying the possible interaction between F-8-F-NH₂ and vasopressin which is also highly localized in posterior pituitary. Interestingly, in Brattleboro rats, F-8-F-NH₂ immunoreactivity in pituitary glands was found to be below limit of detection, while normal level of F-8-F-NH₂ was observed in spinal cords. IN control animals, Long Evans rats, normal level of F-8-F-NH₂ immunoreactivity is present in pituitary glands. The result suggest that there may be an interaction between vasopressin and F-8-F-NH₂.

Distribution of F-8-F-NH₂ containing neuronal cell bodies were studied by immunohistochemical technique using rats which were injected intracerebroventricularly with colchicine. Two strong reactive neuronal groups were found in the rat brain. One of them was located in the periventricular hypothalamic area and whether F-8-F-NH₂ immunoreactivity in posterior pituitary

PROJECT DESCRIPTION

ZO1 MH 01559-07 LPP

originates from these cell bodies is under current investigation. Another prominent cell group was found in the nucleus tractus solitarii. Whether F-8-F-NH₂ containing nerve terminals in the spinal cord originate from the nucleus tractus solitarii will also be investigated.

Significance to the Biomedical Research:

Increasing evidence now suggests the presence of a naturally occurring antiopiate system of peptides that can antagonize morphine effects. Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH₂, which is capable of decreasing the analgesic effect of morphine, may serve as a prototype for this antiopiate system.

Proposed Course of Study:

The proposed course of this study is to investigate 1) function of pituitary F-8-F-NH₂ using Brattleboro rats, 2) release of F-8-F-NH₂ from the spinal cord and the effect of morphine on this release and 3) sources of F-8-F-NH₂ immunoreactive nerve terminals in spinal cords and pituitaries using lesion techniques.

PUBLICATIONS:

Majane, E.A. and Yang, H.-Y.T.: Distribution and Characterization of Two Putative Endogenous Opioid Antagonist Peptides in Bovine Brain. Peptides 8:657-662, 1987.

Roth, B.L., Disimone, J., Majane, E.A. and Yang, H.-Y.T.: Elevation of Arterial Pressure in Rats by Two New Vertebrate peptide FLFPQRF-NH₂ and AGEGLSSPFWSLAAPQRF-NH₂, which are Immunoreactive to FMRF-NH₂ Antiserum. Neuropeptides 10:37-42, 1987.

Panula, P., Kivipelto, L., Nieminen, O., Majane, E.A., and Yang, H.-Y.T.: Neuroanatomy of morphine-modulating peptides. Medical Biology 65:127-135, 1987.

Majane, E.A., Casanova, M.F. and Yang, H.-Y.T.: Biochemical characterization of FMRF-NH₂-like peptides in spinal cords of mammalian species using specific radioimmunoassay. Peptides, In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01577-05 LPP
PERIOD COVERED <u>October 1, 1987 through September 30, 1988</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Characterization of Serotonin Pre- and Postsynaptic Components in NCB-20 Cells</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) <u>J.-M. Cossery, Visiting Fellow, LPP-NIMH</u>		
COOPERATING UNITS (if any) <u>None</u>		
LAB/BRANCH <u>Laboratory of Preclinical Pharmacology</u>		
SECTION <u>Group on Receptor Pharmacology</u>		
INSTITUTE AND LOCATION <u>NIMH, ADAMHA, NTH, Saint Elizabeths Hospital, Washington, D.C. 20032</u>		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We have previously shown that <u>NCB-20</u>, a clonal hybrid of neuroblastoma x fetal Chinese hamster brain cell, expresses serotonin pre-synaptic components. These include a <u>5-HT uptake system</u> and a specific binding sites for <u>imipramine</u> which inhibits the uptake of 5-HT in a competitive manner. Imipramine and related tricyclic antidepressants also activate <u>phosphoinositide hydrolysis</u> by <u>phospholipase C</u>. These effects were nonadditive to those produced by stimulation of muscarinic cholinergic receptors with carbachol and can be desensitized by prestimulation with carbachol. In the present study, we have characterized the post-synaptic serotonin component in the NCB-20 cell, i.e., the 5-HT-sensitive adenylate cyclase, using new specific agonists and antagonists for various classes of 5-HT receptors. Among various agonists examined, only 5-hydroxytryptamine (5-HT), 5-methoxytryptamine (5-MeOT) and the 5-HT₃ agonist 2-methyl-5-hydroxytryptamine (2-Me-5-HT) increased basal accumulation of cyclic AMP by about 100% with an EC₅₀ of about 0.5 μM, 1 μM and 10 μM, respectively. Moreover, we found no effect with the putative agonists for 5-HT_{1A} receptor: i.e., hydroxy-8-N,N-propylamine-2-tetraline (8-OH-DPAT), ipsapirone, buspirone or for 5-HT_{1B} receptor: 1-(3-trifluoromethylphenyl) piperazine (TFMPP), m-chlorophenylpiperazine (m-CPP). The 5-HT effect on basal accumulation was blocked by metergoline but was relatively resistant to 5-HT₃ receptor selective antagonists. A combination of forskolin with 10 μM of 5-HT, 5-MeOT or 2-Me-5-HT produced more than additive effect on cyclic AMP increase, while the agents acting on 5-HT_{1A} and 5-HT_{1B} receptors did not affect the response. Binding data suggest that these cells were virtually devoid of 5-HT_{1A} and 5-HT_{1B} receptor sites. Taken together, it appears that the 5-HT receptor present in this cell line cannot be simply classified as a 5-HT₁ receptor previously described. In fact, this receptor possesses many characteristics of a novel "5-HT₃-like" receptor. </p>		

The understanding of the molecular mechanisms of the interactions between receptors for neurotransmitters in CNS has been hampered by the complexity of brain structures. Part of this complexity arises from the presence of heterogeneous cell populations which include not only neurons but also glial cells. This understanding may be facilitated by the use of a model system of a cloned cell line which contains multiple receptors for neurotransmitters. We have found that NCB-20, a cloned hybrid cell line of mouse neuroblastoma and fetal Chinese hamster brain cell could be such a model system.

We have previously found that, NCB-20 cells possess presynaptic components of 5-HT neurons (Nakaki et al., J. Neurochem. 45:920-925, 1985). These include a 5-HT uptake system and a high affinity binding site for imipramine, a classical tricyclic antidepressant which inhibits 5-HT uptake. Studies by us and others in the CNS have suggested that imipramine is bound to a presynaptic site controlling the uptake of 5-HT in a negative manner. It is of important to mention that the density of imipramine binding site in NCB-20 cells is very high (20 pmol/mg protein). We have recently found that addition of imipramine to NCB-20 cells increased the hydrolysis of phosphoinositide catalyzed by phospholipase C. In these experiments, cells were preincubated with ^3H -myo-inositol to label the endogenous inositol phospholipids and the hydrolysis of phosphoinositide was expressed as the accumulation of ^3H -inositol monophosphate in the presence of lithium. The EC_{50} of imipramine for this response was about 20 μM and the saturating concentration was 100 μM . The increased hydrolysis of phosphoinositide produced by imipramine was nonadditive to that induced by carbachol, a muscarinic cholinergic receptor agonist. However the imipramine response was unaffected by antagonists for muscarinic cholinergic, α_1 -adrenergic, histaminergic H_1 and 5-HT $_2$ receptors. Preexposure of NCB-20 cells to 20 μM imipramine caused a time dependent desensitization to subsequent stimulation with imipramine or carbachol. These data suggest that imipramine "receptors" and muscarinic cholinergic receptors may be coupled to the same pool of phosphoinositide which is the substrate for phospholipase C.

Very recently, we have characterized the post-synaptic serotonin component in NCB-20 cells, i.e. the 5-HT sensitive adenylate cyclase. It is now generally accepted that at least five classes of specific serotonin (5-HT) receptor binding sites exist in nervous tissue. Four of these sites, called 5-HT $_{1A}$, 5-HT $_{1B}$, 5-HT $_{1C}$ and 5-HT $_{1D}$, all exhibit high affinity (nM range K_d) for 5-HT and selected agonists. In contrast, the 5-HT $_2$ receptor has low affinity (μM range K_d) for 5-HT and related agonists, but high affinity for various antagonists. Recently, a new category of 5-HT receptors, called 5-HT $_3$, has been identified in peripheral nerve. These 5-HT receptors, which exhibit low affinity for 5-HT, have now been identified in the brain. (Kilpatrick et al., Nature 330:746, 1987). The ability of the neurohybrid cell line NCB-20 (neuroblastoma x Chinese hamster embryonic brain cell explant) to express 5-HT receptors linked to adenylate cyclase was first shown by MacDermot et al., (Proc. Natl. Acad. Sci. 76:1135, 1979). In a subsequent receptor binding and cAMP formation study, Berry-Kravis and Dawson (1983) found

only one population of 5-HT receptors in this cell line, and concluded that it corresponds to the 5-HT₁ subtype found in rodent brain. They noted, however, that this receptor had a lower affinity for both 5-HT and spiperone, which is in contrast to that of the brain 5-HT₁ receptor. Using newer, more selective 5-HT agonists and antagonists, we undertook a further characterization of the adenylate cyclase - linked 5-HT receptor in NCB-20 cells.

In intact NCB-20 cells, none of the 5-HT_{1A} or 5-HT_{1B} agonists were able to stimulate the production of cAMP. 5-HT itself ($EC_{50} = 0.5 \pm 0.2 \mu M$), 5-MeOT ($EC_{50} = 1.0 \pm 0.1 \mu M$) and the selective 5-HT₃ agonists 2-Me-5-HT ($EC_{50} = 10.0 \pm 0.1 \mu M$) all produced, in a dose dependent fashion, up to a two-fold increase in cAMP accumulation. Furthermore, 8-OH-DPAT neither enhanced nor inhibited the cAMP increase induced by 10 μM of 5-HT. Combined incubation with forskolin (10 μM) and 5-HT, 5-MeOT or 2-Me-5-HT (10 μM) potentiated the forskolin-induced cAMP increase by 2.5 -fold, while the other agonists tested were inactive.

The selective 5-HT₂ antagonist ketanserin (at a concentration up to 100 μM) failed to inhibit the cAMP increase induced by 5-HT. The 5-HT₃ antagonists ICS 205-930 and MDL 72222 (also up to 100 μM) were only able to inhibit 5 to 10% of the cAMP production induced by 10 μM 5-HT. Furthermore, the non-selective antagonist metergoline at 100 μM was found to inhibit completely the cAMP activation induced by 100 μM of 5-HT, with an IC_{50} of approximately 1 μM .

Consistent with the cAMP studies, only 5-HT ($IC_{50} = 0.63 \mu M$) and 5-MeOT ($IC_{50} = 3 \mu M$) were able to displace [³H]-5-HT (80 nM) binding in a specific manner (data not shown). The specific 5-HT_{1A} (8-OH-DPAT, ipsapirone, buspirone) and putative 5-HT_{1B} (TFMPP, m-CPP) receptor agonists did not displace [³H]-5-HT binding, even at concentrations up to 10⁻⁴ M. The selective 5-HT_{1A} radioligand [³H]-8-OH-DPAT showed no specific binding in NCB-20 cells under a variety of binding conditions (temperature: 0°, 24°, 37° C; incubation times: 10-45 minutes; ligand concentration: 1-80 nM).

The current study, utilizing selective 5-HT agonists and antagonists, and combining binding and cAMP accumulation studies clearly demonstrates that the 5-HT receptor in NCB-20 cells linked to adenylate cyclase stimulation is unlikely to be any of the 5-HT₁ type. This finding is strengthened by the absence of binding or biochemical activity for any of the 5-HT_{1A} or 5-HT_{1B} subtype-selective agonists. The insensitivity of the response to ketanserin effectively rules out either a 5-HT₂ or 5-HT_{1C} subtype. Furthermore, it is unlikely that this receptor is of the 5-HT_{1C} or 5-HT_{1D} subtypes, since these are known to be linked to phospholipase C activation and adenylate cyclase inhibition, respectively.

The relatively low affinity of this receptor, as well as the good agonist properties of both 5-MeOT and 2-Me-5-HT for adenylate cyclase stimulation and their inability to inhibit forskolin-induced adenylate cyclase activation, all point to a receptor in the 5-HT₃ class. The relative insensitivity of the 5-HT

PROJECT DESCRIPTION:

Z01 MH 01577-05 LPP

response in NCB-20 cells to the 5-HT₃ antagonist ICS 205-930 supports the notion that the 5-HT₃ receptor may be further divided into subtypes on the basis on the antagonist profile. Thus our results, demonstrating the homogeneity of a 5-HT₃-like receptor in the NCB-20 cell line suggest this system to be a useful model for more intensive pharmacological and biochemical characterization of this receptor subtype.

Our future plans are to further characterize the nature of this novel 5-HT₃ receptor coupled to adenylate cyclase and to study its regulation in response to prolonged agonist stimulation and exposure to differentiation agents. In addition, we would like to study the inter-regulation of 5-HT receptors and other effector-coupled receptors in NCB-20 cells. Since NCB-20 cells are equipped with many different types of neurotransmitter receptors and drug binding sites, this cell line is ideal for the study of multiple receptor interactions at the functional and biochemical levels. The information obtained from this model system may lead to a better understanding of the communication between receptors in the same cell and eventually provide a rational basis for a new approach to the development of therapy for some mental illnesses which are related to abnormalities of receptor-receptor interactions.

PUBLICATIONS:

Cossery, J.-M., Chuang, D.-M. 1988. Characterization of adenylate cyclase-linked serotonin receptor in neurohybrid NCB-20 cells. *FASEB J.* 2, A 1065.

Roth, B. L., Chuang, D.-M. 1988. Multiple mechanisms of serotonergic signal transduction. *Life Science* 41:1051-1064.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02298-03 LPP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Receptor Regulation in Cultured Cerebellum Granule Cells

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation) Onn-Foh Yu, Guest Worker, Ora Dillon-Carter,
Chemist, De-Maw Chuang, Group Chief LPP-NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Group on Receptor Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NTH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:

1.4

PROFESSIONAL:

1.4

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Using primary culture of cerebellar granule cells, we have previously found that these cells possess α -adrenergic, histaminergic H_1 , 5-HT₂ and muscarinic cholinergic receptors coupled to phospholipase C. Prestimulation with each of these receptor agonists was found to cause a time-dependent desensitization to subsequent stimulation with its respective desensitizing agonist. In all cases, the responses mediated by receptors which were not prestimulated remained virtually unchanged, thus indicating homologous desensitization. Biologically active phorbol esters rapidly desensitizes the responses mediated by carbachol, histamine, norepinephrine and serotonin, suggesting that activation and translocation of protein kinase C may play a role in the desensitization of phospholipase C-coupled receptors. Granule cells also express GABA_A and GABA_B receptors. We found that 7-day exposure of these cells to 50 μ M GABA significantly increased the efficacies of carbachol and excitatory amino acids such as quisqualate, L-glutamate, N-methyl-D-aspartate in inducing phosphoinositide (PI) hydrolysis by phospholipase C. This increase was associated with enhancement of the maximal extent of stimulation without significantly affecting the EC₅₀ of these agents. No significant changes in the efficacies and EC₅₀s of norepinephrine and kainate were found after GABA pretreatment. These changes in PI response mediated by muscarinic cholinergic and glutamatergic receptors may underlie some pharmacological effects elicited by long-term treatment with benzodiazepines and barbiturates. In addition, we found that stimulation of GABA_B receptors in granule cells results in inhibition of voltage sensitive calcium uptake and concomitant inhibition of the release of preloaded D-aspartate from granule cells. Thus, cerebellar granule cells express multiple types of cell-surface receptors coupled to various effector systems and is an excellent model for studying the regulation of neurotransmitter receptors.

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Cerebellar granule cells, which are the most numerous and the only known excitatory neurons in the cerebellar cortex, can be dissociated from the tissue of postnatal rats. These primary cultured cells synthesize and release the excitatory transmitter, glutamate. We have attempted to characterize neurotransmitter receptors present in cultured cerebellar granule cells and to investigate the regulation of the signal transduction systems mediated by these receptors.

For the measurement of receptor-coupled phosphoinositide (PI)₃ hydrolysis catalyzed by phospholipase C, granule cells were prelabeled with ³H-inositol and the hydrolysis of labeled phosphoinositide was expressed as the accumulation of ³H-inositol monophosphate (³H-IP₁) in the presence of lithium. We found that addition of 5-HT, NE and histamine caused a 2 to 5-fold increase in the accumulation of ³H-IP₁. Based on sensitivity to receptor antagonists, we concluded that the effects of 5-HT, NE and histamine are mediated by 5-HT₂, adrenergic α_1 and histamine H₁ receptors, respectively. Carbachol, a muscarinic cholinergic receptor agonist, caused a robust (30-40 fold) increase in PI hydrolysis. The carbachol induced effect was blocked by a putative muscarinic M₁ receptor antagonist, pirenzepine, with a moderately low affinity (K_i = 120 nM), suggesting that the classical M₁ receptor is not involved in this receptor signal transduction. Prestimulation with 5-HT, NE, histamine and carbachol was found to cause a time-dependent desensitization to subsequent stimulation with the desensitizing agonist. Thus, prestimulation for 0.5, 4 and 18 hr decreased carbachol response to 87±4, 52±2 and 40±1% of the control, respectively; histamine response to 37±2, 24±2 and 18±2%, respectively; and norepinephrine response to 55±5, 14±1 and 10±1% respectively. In all cases, the responses mediated by receptors which were not prestimulated remained virtually unchanged, thus indicating homologous desensitization. The basal accumulation was markedly enhanced following 0.5 and 4 hr prestimulation but returned to near normal after 18 hr pretreatment. A biologically active phorbol ester, 4- β -phorbol-12-myristate-13-acetate rapidly attenuated basal phospholipase C activity as well as the responses mediated by carbachol, histamine, norepinephrine, and serotonin, suggesting that activation and translocation of protein kinase C may play a role in the desensitization of phospholipase C-coupled receptors.

Granule cells also express phospholipase C-coupled receptors for excitatory amino acids such as L-glutamate, quisqualate, N-methyl-D-aspartate (NMDA) and kainate, and receptors for the major inhibitory amino acid γ -aminobutyric acid (GABA). GABA has previously been reported to regulate the release of preloaded L-glutamate and D-aspartate through activation of low affinity GABA_A receptor and GABA_B receptor, respectively. In addition, inclusion of GABA or GABA mimetics in the culture medium induced neurotrophic effects and the expression of low affinity GABA_A receptor binding sites (Belhage et al., Neurochem. Res. 11:599-606, 1986.) Currently, we are investigating the regulation by GABA of phospholipase C-coupled receptors for neurotransmitters, particularly, excitatory amino acids. We found that exposure of granule cells in culture to 50 μ M GABA for 7 days resulted

in a significant increase in efficacies (50-100%) with no significant change in EC_{50} s of L_2 -glutamate, NMDA, quisqualate and carbachol to accumulate 3H -IP₁ in Mg²⁺ free physiological saline solution. No significant changes in the efficacies and EC_{50} s of NE and kainate were found after GABA pretreatment, thus indicating the selectivity of this modulation. Moreover, the basal PI turnover and the incorporation of 3H -inositol into membrane inositol phospholipid was unchanged by this GABA pretreatment. The effects of GABA on the maximal response of PI turnover mediated by glutamate, NMDA, quisqualate and carbachol were time-dependent, because addition of 50 μ M to the assay medium did not affect PI hydrolysis mediated by these and other stimuli. It is intriguing that the results obtained by pretreatment of granule cells with GABA are reminiscent to some effects that was produced by chronic treatment of animals with benzodiazepines or barbiturates (non-convulsive type). For example, chronic diazepam treatment was reported to increase the Bmax of low affinity GABA_A binding sites (Gallager et al., Eur. J. Pharmacol. 98:159-160, 1984). Chronic benzodiazepines treatment was found to induce changes in muscarinic cholinergic receptor (Popova et al., Gen. Pharmacol. 19:227-231, 1988). Moreover, NMDA antagonist was reported to abolish some of the withdrawal syndrome associated with chronic barbitol treatment. Thus, it seems likely that the selective modulation of carbachol and excitatory amino acid-mediated PI turnover by chronic GABA exposure may underlie some pharmacological basis of the mechanisms of benzodiazepine or barbiturate tolerance. The present study has provided novel information regarding the regulation and some functional role of neurotransmitter receptors present in cerebellar granule cells. In particular, we have made an original observation that chronic GABA treatment can up-regulate the phospholipase C activity stimulated by some excitatory transmitters. This finding should increase our understanding of mechanisms involved in maintaining a functional equilibrium between excitatory and inhibitory neurotransmissions in the CNS and may further provide a new therapeutic basis for treatment of some mental or neurological diseases resulting from a loss of functional balance of the excitatory pathway. Major courses of future investigation are: (1) to study the precise role of protein kinase C in the receptor desensitization process, (2) to determine the level of mRNA for muscarinic cholinergic and other receptors during the course of desensitization and (3) to further characterize the nature of GABA-mediated modulation of neurotransmitter receptors and the physiological and pharmacological significance of this regulation.

PUBLICATIONS:

- (1) Xu, J. and Chuang, D.-M. Serotonergic, adrenergic and histaminergic receptors coupled to phospholipase C in cultured cerebellar granule cells of rats. *Biochem. Pharmacol.* 36:2353-2358, 1987.
- (2) Xu, J. and Chuang, D.-M. Muscarinic acetylcholine receptor-mediated

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02299-03 LPP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Receptor Mediated Phosphoinositide Turnover

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

De-Maw Chuang, Group Chief, Ora Dillon-Carter, Chemist, LPP-NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Group on Receptor Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have studied receptor-mediated phosphoinositide (PI) hydrolysis by phospholipase C in neuroblastoma x brain cell hybrid NCB-20 cells. Stimulation of muscarinic cholinergic, histaminergic and bradykinin receptors was found to increase PI turnover measured by accumulation of H-inositol monophosphate in the presence of lithium. The PI response of muscarinic and histaminergic receptors were highly sensitive to the M₁ receptor antagonist pirenzepine and histamine H₁ receptor antagonist triprolidine, respectively, while the bradykinin response was potently blocked by a bradykinin antagonist B 4881. All three types of PI response appeared to be additive and required unusually high concentration of lithium to display the maximal accumulation of inositol phosphates. The effects mediated by muscarinic and bradykinin receptors were largely dependent upon extracellular calcium but only partially attenuated by pertussis toxin. Preexposure of cells to muscarinic receptor agonist carbachol or bradykinin resulted in homologous desensitization to subsequent receptor stimulation. Phorbol esters also elicited desensitization of these receptor-mediated PI response, suggesting a possible role of protein kinase C in this desensitization process. Phospholipase C activity can also be enhanced by activators of voltage-sensitive sodium channels such as batrachotoxin and veratridine and sodium ionophore such as monensin. Moreover, veratridine can modulate the PI response mediated by muscarinic receptors. NCB-20 cell is an ideal system to study the mechanism, regulation and neurophysiological role of receptor-mediated PI turnover.

It is now well established that neurotransmitters, neuromodulators, hormones and growth-promoting factors exhibit their diverse metabolic and physiological responses by interaction with their selective receptors located on the cell surface. These surface receptors transduce and amplify extracellular signals by the generation of so called second messengers. Among these prominent messengers are inositol trisphosphate (Ins-P_3) and 1,2-diacylglycerol which are formed by hydrolysis of membrane-bound phosphoinositides (PI) in response to receptor stimulation. Ins-P_3 mobilizes intracellular calcium from nonmitochondrial stores, while diacylglycerol activates a calcium and phospholipid-dependent protein kinase C. These dual messengers act synergistically to trigger an array of physiological processes including neuronal membrane excitability, synaptic transmission, cellular metabolism, muscle contraction, sensory transduction, motility, growth and DNA synthesis. The brain and related neural tissues are particularly enriched in phospholipase C, the enzyme catalyzing the hydrolysis of PI and very active in metabolizing this inositol phospholipid. Hence, a study of the receptor-mediated PI hydrolysis in neuronal tissues is warranted.

The study of receptor-mediated PI hydrolysis in brain tissues has been complicated by the presence of an extremely heterogeneous cell population and sometimes hampered by a relatively small signal of activation. In this report, we have used the NCB-20 cell line which is a neurotumor derived from fusion between mouse neuroblastoma and Chinese hamster 18-day embryonic brain cell, to study the mechanisms of neurotransmitter receptors-coupled PI hydrolysis. NCB-20 cells were grown to confluency and then incubated overnight with ^3H -myo-inositol to label the endogenous PI. The turnover of PI was measured by the accumulation of ^3H -inositol monophosphate (IP_1) in the presence of lithium, which has been shown previously to inhibit the activity of inositol monophosphate phosphatase.

Among nearly 20 neurotransmitters and neuromodulators examined, only carbachol, histamine and bradykinin were able to increase the accumulation of ^3H - IP_1 by 5-, 3- and 10-fold, respectively. The EC_{50} of carbachol was about $50\ \mu\text{M}$ and the effect was potently blocked by a putative muscarinic M_1 receptor antagonist pirenzepine with a K_i of 25 nM. Bethanecol and pilocarpine were partial agonists in promoting the response, whereas oxotremorine, McN-A-343 and AHR-602 were virtually inactive. The amounts of inositol bis- and trisphosphates were also increased, but these increases were faster in time course and smaller in magnitude when compared with the parameters of IP_1 . The histamine response ($\text{EC}_{50}=20\ \mu\text{M}$) was blocked by two histamine H_1 receptor antagonists, triprolidine and phenhydramine, but was unaffected by a histamine H_2 receptor antagonist, cimetidine. The effect of bradykinin ($\text{EC}_{50}=20\ \text{nM}$) was potently and selectively inhibited by a bradykinin antagonist, B 4881 (D-Arg-Hyp 3 , Thi 5,8 , D-phe-bradykinin) with a K_i of 10 nM. The effects of bradykinin, histamine and carbachol were additive to each other at concentrations that displayed maximal activation of PI turnover.

The effects of bradykinin and carbachol were largely dependent on the presence of calcium in the extracellular medium and were partially attenuated by pretreatment

with pertussis toxin, suggesting that more than one type of GTP binding protein is involved in these receptor-mediated events. The responses of both bradykinin and carbachol were desensitized by preexposure of NCB-20 cells to bradykinin and carbachol, respectively. This desensitization was homologous and involved a loss of the maximal extent of receptor-activated PI turnover. Biologically active phorbol ester such as phorbol myristate acetate (PMA) and phorbol dibutyrate (PDB) markedly attenuated the carbachol and bradykinin-mediated response. The basal activity of phospholipase C was also significantly inhibited by these phorbol esters. These data imply that either phospholipase C or some regulatory protein(s) for this enzyme may be the substrate for a protein kinase C which is activated by phorbol esters. We also found that the concentration of lithium required to maximally increase the receptor-activated IP_1 accumulation in NCB-20 cells was about 60-80 mM which was about 10-times greater than those found in brain slices and other neurotumor cell lines such as NG108-15 neurohybrid. This requirement of high concentration of lithium for eliciting IP_1 accumulation may suggest that NCB-20 cells express an unusual form of inositol-1-phosphatase which is more resistant to inhibition of its enzymatic activity by lithium.

Very recently we have studied the effects of two sodium channel activators veratridine and batrachotoxin (BTX) and a sodium ionophore, monensin on PI turnover in NCB-20 cells. Veratridine, BTX and monensin caused a dose-dependent increase in basal accumulation of 3H - IP_1 . At respective concentrations of 50, 0.5 and 50 μM , these three agents stimulated PI turnover to 126, 171 and 222% of the control. The percentage of stimulation induced by these agents was unaffected by replacing NaCl in the medium with choline chloride or isotonic sucrose, suggesting that influx of sodium is not a prerequisite for this activation. Tetrodotoxin (TTX) blocked effects of BTX and veratridine but not the stimulation by monensin in normal and sodium-free media with an IC_{50} of approximately 20 nM. The stimulatory effects of these three agents were largely dependent on the presence of extracellular calcium, as evidence by more than 80% loss of the activation by replacing calcium with EGTA. The effect of BTX was inhibited by micromolar concentrations of nimodipine, while the monensin-induced stimulation was unaffected by this voltage-sensitive calcium channel blocker. Quabain, an inhibitor of Na^+ , K^+ -ATPase, did not alter the turnover of PI. Taken together, these results strongly suggest that binding of sodium channel activators and monensin to intact cells promotes the activity of phospholipase C in a calcium-dependent manner, but this effect may not involve Na^+/Ca^{2+} exchange mechanisms. BTX and monensin also induced greater than additive effect on carbachol-stimulated 3H - IP_1 accumulation. The effect of BTX on carbachol response was TTX-sensitive and associated with 40% increase in the maximal stimulation and a similar extent of decrease in the EC_{50} of carbachol. Veratridine provoked strikingly different effects on carbachol-dependent PI turnover in cells with low and high passage numbers. In young cell culture, veratridine produced a nearly additive effect. By contrast, in old culture veratridine markedly inhibited the stimulation induced by subsaturating concentrations of carbachol. This veratridine-induced suppression was TTX-insensitive and was due to 8 to 10-fold

increase in the EC_{50} of carbachol with no change in the maximal effect. Displacement of 3H -n-methylscopolamine binding to intact cells with carbachol revealed that inhibition of carbachol binding to cell-surface receptors cannot explain the inhibitory effect of veratridine. PI turnover stimulated by histamine and bradykinin was not suppressed by veratridine, thus indicating the specificity of this modulation.

Neurotransmitters receptor-mediated PI turnover has been implicated in synaptic transmission, axonal regeneration and the process of learning and memory. In the present study, we showed that NCB-20 cells express muscarinic cholinergic, histaminergic H_1 and bradykinin receptors coupled to phospholipase C. These receptor responses are regulated by phorbol esters and pertussis toxin and can be desensitized by prestimulation with their receptor agonist. These novel information has increased our understanding of the regulatory mechanisms of neurotransmission mediated by receptor-activated PI turnover. The presence of an unusual form of inositol-1-phosphatase in NCB-20 cells may be of great importance in understanding the mechanisms of action of lithium. Since inositol-1-phosphatase is a possible site of action of lithium in the treatment of manic depression, our finding that high lithium is required to inhibit the activity of this phosphatase may have implication for some clinical cases in which bipolar depressive patients are not beneficial from treatment with lithium. Moreover, we found that the activity of phospholipase C in NCB-20 cells can be enhanced by activation of voltage-sensitive sodium channel. This may provide mechanisms by which the hydrolysis of PI can be activated by depolarization of neurons. In addition, our preliminary results show that tricyclic antidepressants and the antimanic drug carbamazepine can elicit an increase in inositol phosphate accumulation in relatively high concentration ranges. Our future plans are to study the effects chronic exposure of NCB-20 cells to therapeutic concentrations of tricyclic antidepressants, carbamazepine and lithium on receptor-mediated PI turnover. These studies may lead to better understanding of the molecular mechanism whereby these drugs display their therapeutic effects and might eventually lead to discovery of therapeutic modalities that can prevent, alleviate or cure the malfunction in the homeostatic mechanism involved in the disease state.

PUBLICATIONS:

Chuang, D.-M. Carbachol-induced accumulation of inositol-1-phosphate in neurohybridoma NCB-20 cells: Effect of lithium and phorbol esters. *Biochem. Biophys. Res. Commun.* 136:622-629, 1986.

Chuang, D.-M. and Dillon-Carter, O. Characterization of bradykinin-induced phosphoinositide turnover in neurohybrid NCB-20 cells. *J. Neurochem.* (in press).

Zhu, X.-Z. and Chuang, D.-M. Differential regulation of opioids, α_2 -adrenergic and muscarinic acetylcholine receptors in NCB-20 cells by butyrate and dibutyl cAMP.

PROJECT DESCRIPTION

ZO1 MH 02299-03 LPP

J. Neurochem. 50:17-26, 1988.

Chuang, D.-M. Modulation of phospholipase C activity by activators of sodium channel in neurohybrid NCB-20 cells. FASEB J. 2:A 1380, 1988.

Chuang, D.-M. Neurotransmitter receptor and phosphoinositide turnover. Ann. Rev. Pharmacol. and Toxicol. Vol 29. (In press), 1989.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02301-03 LPP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functional Role of Adrenal NPY

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

K. Shimoda, Visiting Associate, H.-Y.T. Yang, Section Chief LPP-NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuropeptides

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

High concentrations of neuropeptide Y (NPY) have been found in many chromaffin cells in adrenal glands of various species. Previously, we have found that, in addition to authentic NPY, there is a NPY-like peptide in chromaffin cells of bovine adrenal glands. Interestingly, in the rat, this NPY-like peptide is present in the adrenal gland of the old rat but not in that of the young rat. This NPY-like peptide has now been purified to homogeneity and characterized by sequencing, other chemical analysis and carboxypeptidase hydrolysis. A high degree of homology with NPY was observed and final confirmation of the structure determination is in progress. In order to study whether the NPY-like peptide may have a modulatory role in catecholamine secretion from chromaffin cells, we have now established the primary culture of rat adrenal chromaffin cells. The characterization of the rat adrenal chromaffin cell during the course of the culture is in progress.

Significance to Biomedical Research:

A novel NPY-like peptide has been isolated from bovine adrenal glands. This peptide is present in the adrenal gland of the old rat in significant quantity but not in that of the young rat. Study on biological activities of this peptide may provide a new direction to study the adrenal function of aged animal.

1079

PROJECT DESCRIPTION:

ZOL MH 02301-03 LPP

High concentrations of neuropeptide Y (NPY) have been found in many catecholamine containing chromaffin cells in adrenal glands of various species. previously, we have found that besides NPY there is an additional NPY-like peptide in adrenal glands of bovine and rat. Interestingly, this NPY-like peptide is present in the adrenal gland of the older rat but not in that of the younger rat. Using bovine adrenal chromaffin cells and retrogradely perfused bovine adrenal glands, we found that the NPY-like peptide can be released by stimulation with 56 mM KCl or acetylcholine. In order to further explore the biological implication of the appearance of the NPY-like peptide in the adrenal glands of older rats, we have now purified the NPY-like peptide to homogeneity from bovine adrenal chromaffin granules by BioGel P-10 chromatography followed with successive steps of high pressure liquid chromatography. The purified peptide was characterized by sequencing, other chemical analysis and protease hydrolysis. A high degree of homology with NPY was observed. The NPY-like peptide was found to have free carboxyl group at its c-terminal as it was readily hydrolyzed by carboxypeptidase A treatment. The final confirmation of the structure by comparing with synthetic peptide is in progress.

In order to study whether the NPY-like peptide may have a modulatory role on the function of adrenal catecholamines especially the secretion of catecholamines, we have begun to establish a primary culture of rat adrenal chromaffin cells. The results to date indicate that it is possible to culture the rat adrenal chromaffin cells. The characterization of this primary culture of rat chromaffin cells, in terms of their catecholamine and NPY contents and release of these two constituents, is in progress.

Significance to Biomedical Research:

The NPY-like peptide is present only in the adrenal gland of the old rat in significant quantity. Study on biological activities of this newly isolated peptide may provide a new direction to study the adrenal function in aged animals.

Proposed Course of Study:

We plan to (1) finalize the structure of the NPY-like peptide and (2) study biological activities of the NPY-like peptide.

Publications:

1. Higuchi, H., Costa, E. and Yang, H.-Y.T.: Neuropeptide Y inhibits the nicotine mediated release of catecholamine from bovine adrenal chromaffin cells. J. Pharmacol. Exp. Ther. 244:468-474, 1988.
2. Hexum, T.D., Majane, E.A., Russett, L.R. and Yang, H.-Y.T.: Neuropeptide Y release from the adrenal medulla after cholinergic receptor stimulation. J. Pharmacol. Exp. 243:927-930, 1988.

PROJECT DESCRIPTION:

Z01 MH 2301-03 LPP

3. Higuchi, H., Yang, H.-Y.T. and Sabol, S.L.: Rat neuropeptide Y precursor gene expression: mRNA structure, tissue distribution, and regulation by glucocorticoids, cyclic AMP, and phorbol ester, J. Biol. Chem. 263:6288-6295, 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02378-02 LPP
PERIOD COVERED <u>October 1, 1987 through September 30, 1988</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Histochemical Localization of Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH₂ Immunoreactivity in Mammalian CNS</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) <u>T. Salminen, Visiting Fellow, H.-Y.T. Yang, Pharmacologist LPP-NTMH</u>		
COOPERATING UNITS (if any) <u>C.-H. Lee, Naval Medical Research Institution; Bethesda, MD</u>		
LAB/BRANCH <u>Laboratory of Preclinical Pharmacology</u>		
SECTION <u>Section on Neuropeptides</u>		
INSTITUTE AND LOCATION <u>NTMH, ADAMHA, NTH, St. Elizabeths Hospital, Washington, D.C. 20032</u>		
TOTAL MANYEARS: <u>0.5</u>	PROFESSIONAL: <u>0.5</u>	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Previously, we have isolated and chemically characterized a <u>FMRF-like peptide</u> of bovine brain. This peptide, <u>phe-leu-phe-gln-pro-gln-arg-phe-NH₂ (F-8-F-NH₂)</u>, can decrease analgesic effect of morphine and is highly localized in spinal cord and periaqueductal gray areas. In this study, the locations of F-8-F-NH₂ in periaqueductal gray areas of rat and bovine brains were investigated immunohistochemically using <u>polyclonal</u> and <u>monoclonal antibodies</u>. The polyclonal antiserum cross-reacts slightly with NPY (<0.1%), while the monoclonal antibody is devoid of such cross-reactivity. F-8-F-NH₂ immunoreactivity is localized in nerve fibers running vertically throughout periaqueductal gray area. In horizontal sections F-8-F-NH₂ <u>immunoreactive nerves</u> form dense networks in posterolateral periaqueductal gray areas. F-8-F-NH₂ immunoreactive cell bodies were not detected in the periaqueductal gray areas even in the rats which were treated with colchicine to block axonal transport. The immunostaining was abolished by preabsorption with F-8-F-NH₂ but not by NPY which is structurally similar to NPY in its c-terminal dipeptide. The results of this study indicate that there are specific F-8-F-NH₂ containing nerve fibers in periaqueductal gray areas and they seem to originate from other parts of the brain.</p>		

PROJECT DESCRIPTION:

Z01 MH 02378-02 LPP

Previously, we have isolated and chemically characterized a FMRF-NH₂-like peptide of bovine brain. This peptide, phe-leu-phe-gln-pro-gln-arg-phe-NH₂ (F-8-F-NH₂), can attenuate the morphine induced prolongation of tail flick latencies in the rat when injected intraventricularly. In bovine CNS, F-8-F-NH₂ was found to be highly localized in dorsal spinal cord and periaqueductal gray areas by radioimmunoassay. In order to further explore the functional role of F-8-F-NH₂, monoclonal antibodies to F-8-F-NH₂ were developed and characterized by radioimmunoassay techniques. The results suggest that these monoclonal antibodies are capable of differentiating F-8-F-NH₂ or F-8-F-NH₂ like peptides from neuropeptide Y (NPY) which is structurally similar to F-8-F-NH₂ or FMRF-NH₂ in its c-terminal sequence. In fact, it is difficult to differentiate FMRF-NH₂-like peptides from NPY immunohistochemically with some polyclonal antisera (Sasek and Elde, 1985). In this study, the location of F-8-F-NH₂ in PAG area of rat and bovine brain was investigated immunohistochemically. Both polyclonal and monoclonal antibodies were used and compared. F-8-F-NH₂-IR is localized in nerve fibers running vertically throughout PAG area. In horizontal sections, F-8-F-NH₂ immunoreactive nerves form dense networks in posterolateral PAG. In contrast to normal animals, rats pretreated with colchicine showed only a few nerve fibers but even then no cell bodies were found. The immunostaining was abolished when the antiserum was preabsorbed with F-8-F-NH₂ but not with NPY. Monoclonal antisera produced similar staining pattern as polyclonal antisera. The F-8-F-NH₂ immunoreactivity in bovine PAG was characterized by HPLC coupled with RIA and the major immunoreactivity was identical to F-8-F-NH₂. The results of this study indicate that there are specific F-8-F-NH₂ containing nerve fibers in PAG areas and they seem to originate from other parts of the brain.

Significance to the Biomedical Research:

F-8-F-NH₂ was found to be highly localized in periaqueductal gray by the radioimmunoassay. This study indicates the presence of F-8-F-NH₂ in nerve fibers in the periaqueductal gray, the area important for the mediation of antinociception. The antiopiate property of F-8-F-NH₂ and result of this study further suggest that F-8-F-NH₂ may have a role in antinociception and furthermore in development of opiate tolerance.

Proposed Course of Study:

The project is to be terminated due to the departure of principle investigator.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02316-03 CRDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Teaching the Wisconsin Card Sort of Schizophrenic Patients

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Terry Goldberg, Ph.D., Special Expert, CBDB, NIMH

Daniel R. Weinberger, M.D., Chief, CBDB, NIMH, Karen F. Berman, Staff Psychiatrist, CBDB, NIMH;
Marvin Podd, Ph.D., O'Malley Division, Saint Elizabeths Hospital

COOPERATING UNITS (if any)

O'Malley Division, Saint Elizabeths Hospital

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL MAN-YEARS:

.33

PROFESSIONAL:

.33

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02351-02 CBDB
PERIOD COVERED October 1, 1987 through September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pathology of Selected Central Nervous System Degenerative Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Dr. M. F. Casanova, Neurologist and Neuropathologist, NIMH Dr. D. Price, Director Neuropathology Laboratory, Johns Hopkins University Dr. R. Struble, Assistant Professor of Neuropathology, Johns Hopkins University Dr. P. Whitehouse, Associate Professor, Case, Western University Dr. J. Kleinman, Deputy Director, CBDB-NIMH		
COOPERATING UNITS (if any) Clinical Brain Disorders Branch, NIMH; Neuropsychiatry Branch, NIMH		
LAB/BRANCH Clinical Brain Disorders Branch		
SECTION Section on Neuropathology Studies		
INSTITUTE AND LOCATION NIMH, Neuroscience Center at Saint Elizabeths		
TOTAL MAN-YEARS: 1	PROFESSIONAL: 3	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Selected neuropsychiatry conditions <u>Alzheimer's Disease</u> (AD), <u>Schizophrenia</u> (SC), and <u>Dystonia musculouem deformons</u> (DMD) were studied with quantitative anatomical techniques in order to establish clinico-pathological correlates and elucidate pathogenesis. Some of the structures incriminated in our research were further studies during gestation to discern possible developmental results which could rise to these neuropsychiatric conditions. A. In AD: We described involvement of the <u>noradrenergic system</u> in <u>hippocampal pathology</u> and suggested a possible relationship to the dystonia observed in this condition. B. In SC: We reviewed the literature and current status of the Neuropathology of schizophrenia and examined alternate methods for the quantitative assessment of <u>gliosis</u> . C. In DMD: We provided the first report of pathology involving various brainstorm structures. D. In Development: We examined the "strisome" pattern of innervation of baboons at several stages of gestation and adulthood the significance of the project lies in the identification of pathological correlates to those neuro-psychiatric conditions as a first step towards the discovery of their etiology and possible therapeutic interventions.		

Objectives: To examine autopsy tissue for abnormal neuronometrics, immuno-cytochemical and autoradiographic changes in the brains of Alzheimer's Disease (AD), Schizophrenia (SC), and dystonia musculorum Deformans (DMD) patients. The areas to be studied represent possible sites of pathology as suggested by the clinical manifestations of the disease. Hopefully, delineation of pathological changes will provide important clues as to the pathophysiology of the disease and permit eventual investigations of their etiology and possible therapeutic interventions.

Methods Employed: Brains are collected from the DC medical examiners and processed accordingly for computer image analysis, immunocytochemistry and/or autoradiography. The emphasis on quantitative techniques and the combined use of anatomical and neurochemical studies.

- 1) Computer assisted image analysis. Deals with problems of quantitative microscopy in the assessment of different neuronal population.
- 2) Immunocytochemistry-Combining the standard method of the Stenbeger's with acrlien as a fixative provides the opportunity to qualitatively describe the presence and distribution of antigen in tissue.
- 3) Autoradiography: Computer assisted densitometric analysis of grain density on film is used to define receptor concentrations in specific anatomical areas.

New Findings:

- 1) In AD: We describe the normal innervation of the hippocampus by the noradrenergic system and how it was affected in aging and AD. These abnormalities may account for the disruption of the blood brain barrier observed in AD patients.
- 2) In DMD: We provided the first pathological correlate for primary (hereditary) forms of dystonia and a possible explanation for both sleep and EEG abnormalities.
- 3) In SC: We reviewed a published report regarding astrocytosis in this illness but could not confirm their findings.
- 4) In Development: We confirmed the cholinergic nature of "Striosoners" and described similar patterns of innervation during development for adrenergic and dopaminergic receptors.

Significance to Mental Health Research: Delineation of these pathological changes in SC, AD and DMD provides important clues as to the pathophysiology of these diseases and will hopefully, permit eventual investigations of their etiology and possible therapeutic interventions.

Proposed Course of Project: We will concentrate most of our research effort in schizophrenia. The use of computer assisted image analysis will allow quantitation of pigments (e.g.: lipofuscin and iron) and provide measurements of disarray or tangledness in the pyramidal cells of the hippocampus. At the same time we will combine anatomical and neurochemical techniques in the study of structures of the limbic system with emphasis on the entorhinal cortex.

A. Last year's quoted in press:

Zweig RM, Whitehouse PJ, Casanova MF, Walker LC, Jankel WR and Price DL: Loss or pedunculo-pontine neurons in progressive supranuclear palsy. *Ann. Neurol.*, *Ann Neurol* 22:18-25, 1987.

Struble RG, Powers RE, Casanova MF, Brown EC, Kitt CA and Price DL: Neuro-peptidergic systems in plaques of Alzheimer's disease. *J. Neuropathol. Exp. Neurol.* 46:567-584, 1987.

Casanova M, Stevens J, Bigelow L: Gliosis in schizophrenia, *Biol. Psychiatry* 22:1172-1173, 1987.

Lowenstein PR, Slesinger PA, Singer HS, Walker LC, Casanova MF, Price DL and Coyle JT: An autoradiographic study of the development of 3H-hemicholinium-3 binding sites in human and baboon basal ganglia: A marker for the sodium dependent high affinity choline uptake system. *Dev. Brain Res.*, in press.

B. This year's:

Price DL, Struble RG, Powers RE, Casanova MF, Kitt CA, Whitehouse PJ, and Cork LC: Neuropathological correlates of dementia in Alzheimer's disease. In: *Proceedings of the IVth World Congress of Biological Psychiatry*. New York, Elsevier, 1987.

Powers RE, Struble RG, Casanova MF, O'Connor DT, Kitt CA and Price DL, Innervation of human hippocampus by noradrenergic systems normal anatomy and structural abnormalities in aging and in Alzheimer's disease. *Neuroscience*, in press.

Zweig RM, Hedreen JC, Jankel WR, Casanova MF, Whitehouse PJ, and Price DL, Pathology in brainstem regions of individuals with primary dystonia. *Neurology* 38: 702-706, 1988.

Slesinger PA, Lowenstein PR, Walker LC, Casanova MF, Price DL, Coyle JT and Singer HS: The development of B1 and B2 adrenergic receptors in baboon brain: an autoradiographic study using [125I] iodocyanopindolol. *J Comp Neurol*, in press.

Kleinman J, Casanova MF, Yang H-YT: The Neuropathology of Schizophrenia. *Schizophrenia Bull.*, in press

Majane EA, Casanova MF, H-YT: Biochemical characterization of FMRF-NH₂ like peptides in spinal cords of various mammalian species using specific radioimmunoassays, *Peptides*, in press.

Wagner HN, Weinberger Dr, Kleinman JE, Casanova MF, et.al.: Neuroimaging and neuropathology panel, Report to the national advisory mental health council on a national plan for research on schizophrenia, *Schizophrenia Bull.*, in press.

Casanova MF: Brain Death: a personal perspective, *Bol. Assoc. Med. P.R.*, 80(50): 173, 1988.

Stevens JR, Casanova, MF: Is there a neuropathology of schizophrenia?
(Editorial), Biol Psy 24:123-128, 1988.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02352-03 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Prefrontal Cortical Modulation of Subcortical Dopamine Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

George Jaskiw, M.D., Visiting Scientist, CBDB, NIMH

Farouk Karoum, Ph.D., Research Chemist, NPB, NIMH; William Freed, Ph.D., Chief, Preclinical Neurosciences Section, NPB, NIMH; Joel E. Kleinman, M.D., Ph.D., Chief, Section on Neuropathology, CBDB, NIMH; Daniel R. Weinberger, M.D., Chief, CBDB, NIMH

COOPERATING UNITS (if any)

Neuropsychiatry Branch, NIMH

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies; Molecular Neuropsychiatry; Preclinical Neurosciences

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL MAN-YEARS:

8

PROFESSIONAL:

1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have continued to examine the relationships between cortical and subcortical systems, particularly between catecholamines in the prefrontal cortex (PFC) and the nucleus accumbens and corpus striatum. Using sensitive measures of activity we have determined that following chemical lesioning of efferents from the prefrontal cortex rats develop a state both of basal hyperactivity and hyperexploration. As recovery occurs, first the hyperactivity and then the increased exploratory behavior decline to near baseline levels by one month after the lesion.

Neurochemical changes occur in parallel with those in activity. Following chemical destruction of PFC efferents, dopamine turnover in the PFC, the cingulate cortex and in the striatum are increased. These changes disappear by one month after the lesion.

It appears that the prefrontal cortex can modulate catecholamine turnover and some associated behaviors both in other cortical and in subcortical areas.

Project Description:

Objectives: The modulation of dopamine turnover in the brain subcortical remains poorly understood. Some investigators suggest that the various dopaminergic terminal fields feed back on each other to determine final dopaminergic tone. By destroying individual components of this network and assessing behavioral and biochemical changes we probed the nature of the modulation.

Methods Employed: Stereotactic neurotoxic lesions were made in the PFC of male rats. After various recovery intervals some rats were tested for baseline exploratory activity; others were repeatedly tested in the same environment. Animals were then sacrificed and cerebral regions were dissected catecholamine metabolite analysis.

Major Past Findings: Amphetamine and apomorphine induced behaviors and stereotypy did not appear to be altered 2-6 weeks after PFC destruction.

New Findings: Chemical destruction of PFC efferents produces a transient hyperexploratory/hyperactive state as well as indices of increased catecholamine turnover in the PFC, cingulate cortex and the striatum. These returned to baseline by one month.

These findings suggest that neurons intrinsic to PFC provide an inhibitory influence on DA neurons projecting to PFC, cingulate and striatum, indicating that one cortical terminal field of DA neurons can influence DA turnover in other cortical and subcortical areas.

Significance to Mental Health Research: Considerable evidence implicates dysfunction of both cortical and subcortical dopamine systems in schizophrenia. The nature of the dysfunction is not known. Our work suggests that pathophysiology of a discrete cortical area brain could cause wide ranging alterations in dopaminergic tone. If this is the case in schizophrenia then specific therapeutic maneuvers aimed at the area of primary pathophysiology could be developed.

Proposed Course of Project: Several paradigms will be pursued. 1) pharmacological agents as well as physiological stressors which activate the PFC dopamine systems will be administered at various times after PFC destruction 2) the development of the selective stress response of PFC DA neurons will be examined in normal rats of different ages 3) homeostatic mechanisms in the dopamine system will be evaluated following neonatal and intrauterine insults to those systems.

Publications:

Jaskiw GE, Freed WJ, Farouk K, Kleinman JE, Weinberger DR: Locomotor activity following ibotenic acid lesions of the medial prefrontal cortex in the rat. Brain Res (in submission).

Jaskiw GE, Farouk K, Freed WJ, Kleinman JE, Weinberger DR: Changes in catecholamine turnover following ibotenic acid lesions of the medial prefrontal cortex in the rat. Synapse (in submission).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02353-03 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cranial Asymmetries and the Reliability of the International 10-20 System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Michael Myslobodsky, M.D., CBDB, NIMH

Richard Coppola, D.Sc., CBDB, NIMH; Craig Karson, M.D., CBDB, NIMH; David Daniel, M.D., CBDB, NIMH;
Daniel R. Weinberger, M.D., Chief, CBDB, NIMH

COOPERATING UNITS (If any).

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL MAN-YEARS:

1

PROFESSIONAL:

3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02354-02 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Amphetamine and Frontal Lobe Functioning in Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Terry Goldberg, Ph.D., Special Expert, CBDB, NIMH

Joel E. Kleinman, M.D., Ph.D., Deputy Chief, CBDB, NIMH; Llewellyn B. Bigelow, M.D., Sen. Staff Scientist, CBDB, NIMH

COOPERATING UNITS (If any)

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies; Neuropathology

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL MAN-YEARS:

.25

PROFESSIONAL:

.25

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Research on the effects of amphetamine in schizophrenia is conflicting. It is known that amphetamine may cause paranoid psychosis. On the other hand, transitory improvement in some patients given acute doses of amphetamine has been reported. It is reasonable to speculate that negative symptoms of schizophrenia, such as anergia, lack of motivation, flattened affect, and poor planning may be amenable to such treatment. In addition, patients with schizophrenia generally do poorly on neuropsychological tests thought to assess frontal lobe functioning. We therefore propose a pharmacological strategy involving the administration of amphetamine in the hope that this drug may reverse some of the abnormalities found in chronic schizophrenia, whether they be symptomatic or cognitive. However, to reduce the risk of worsening psychotic symptoms with dopamimetic therapy, all patients will be maintained on a neuroleptic.

Project Description:

Objectives: It is hypothesized that administration of amphetamine will increase physiological activation in the prefrontal neural system that mediates cognitive activities involving planning, working memory, and responsivity to external stimuli (including reward). In addition, subcortical structures that may be affected by amphetamine might manifest activity in rotational behavior or eye blinking.

Methods Employed: A double blind cross-over design is used. Subjects in group one will receive placebo first and five days later an acute oral dose of .25mg/kg dextroamphetamine elixir. Subjects in group two will receive .25mg/kg dextroamphetamine orally and five days later placebo. Following administration, rotational behavior of patients will be assessed by rotometer. One hour after administration of medication or placebo patients will be tested on the Wisconsin Card Sort test, Selective Reminding, CPT, and other neuropsychological tests. In addition, patients' affect will be assessed.

Major Past Findings: The study is in progress.

Significance to Mental Health Research: The study may provide findings about the notion that the prefrontal system in chronic schizophrenia is underactive. In addition, amelioration of such underactivation might have implications for treatment (though not necessarily with amphetamine).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02355-02 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Autism: A Study of Cerebrophysiology, Neuroanatomy and Neuropsychology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Terry Goldberg, Ph.D., Special Expert, CBDB, NIMH

Daniel R. Weinberger, M.D., Chief, CBDB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL MAN-YEARS:

.5

PROFESSIONAL:

.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Few studies have employed multimodal measurements in the same autistic individual, thus considerably reducing their power. The current proposal will attempt to use cerebral blood flow, magnetic resonance imaging, and neuropsychology to provide convergent evidence as to the source of the deficit in autism. In this study, autistic individuals would be presented with cognitive tasks and affect laden tasks in order to challenge various brain regions. Activation will then be correlated with brain structures that were imaged on MR scans as well as results of neuropsychological tests tapping attention, memory, procedural abilities, language, and visual spatial functions. By utilizing autistic individuals with savant skills, we hope not only to gain greater understanding of the neurobiology of exceptional performance, but increased "signal-to-noise" ratio in the autistic population. Autistic Individuals without savant skills and mentally retarded individuals, as well as normals, will serve as controls.

Project Description:

Objectives: By using cerebral blood flow, magnetic resonance imaging and neuropsychology, we hope to provide converging evidence as to the region or regions of disorder in autism. Furthermore, we hope to gain greater understanding of qualitative and quantitative characteristics associated with savant skills, a domain that has implications for cognitive science, neuropsychiatry, and the study of exceptional abilities.

Major Past Findings: The study is in progress.

Significance to Mental Health Research: We hope the study will have implications for treatment. By carefully delineating areas of normality, hyperactivity or underactivity in the brain, we hope to be able to suggest pharmacological treatments or perhaps even psychoeducational treatments that will ameliorate the disorder. Of course, we hope also, that it permits us to better characterize the nature of autism from a pathophysiological viewpoint.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02356-02 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Procedural and Problem Solving Abilities in Schizophrenic Patients

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Terry Goldberg, Ph.D., Special Expert, CBDB, NIMH

Jean St.-Cyr, Ph.D., Visiting Scientist, Neuropsychology IRP, NIMH; Daniel R. Weinberger, M.D., Chief, CBDB, NIMH

COOPERATING UNITS (if any)

Neuropsychology Laboratory, NIMH

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL MAN-YEARS:

.25

PROFESSIONAL:

.25

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Certain tasks that come under the rubric of procedural may be learned by subjects with various organic disorders who otherwise have marked difficulty learning new information. One such task is called Tower of Hanoi. It involves moving discs from one peg to another. As well as a procedural component, it also involves problem solving and has been used as a model for conscious problem solving in artificial intelligence. It is thought that the neural system which mediates procedural performance (the basal ganglia) is distinct from the system that is involved in higher level problem solving (prefrontal) or everyday long term memory for items (diencephalic and medial temporal). Two versions of the Tower of Hanoi were administered to schizophrenic patients as well as a control task that involved visual spatial processing. In addition, efforts were made to teach both tasks to patients who experienced difficulty.

Project Description:

Objectives: We wish to assess performance in the procedural realm and, if possible, compare it to higher level problem solving that may involve visual spatial components and strategy in planning. The systems at the neural level involved in all three may be distinct. Therefore, direct comparisons might allow one to infer regions of dysfunction and integrity in schizophrenia. In addition, teaching the tasks might shed light on whether deficits, if found, are valid and reflect competence rather than performance. Such a strategy had been used with a prior study of the Wisconsin Card Sorting Test.

Methods Employed: Normal subjects and patients in the William A. White program who suffered from schizophrenia, participated in the study. Patients were receiving drugs. Patients and normal control subjects received a three disk version of the Tower, a four disk version of the Tower, and the block design task from the WAIS-R. Also, subjects were administered multiple trials of the Tower over four days to assess learning.

Major Past Findings: The study has been completed. Patients performed at least as well on the more difficult four disk version as on the three disk version relative to controls. This suggests their basal ganglia may be more intact than their prefrontal system.

Significance to Mental Health Research: The study may improve our understanding of the relationship between cortical and subcortical structures responsible for various aspects of problem solving. This study implicated a specific neural system, the prefrontal, but not the basal ganglia to the same degree.

Publications:

Goldberg TE, Weinberger DR: Probing prefrontal function in schizophrenia with neuropsychological paradigms. Schizophrenia Bulletin, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02357-03 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Recall and Recognition Memory in Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Terry Goldberg, Ph.D., Special Expert, CBDB, NIMH

Daniel R. Weinberger, M.D., Chief, CBDB, NIMH; Neil H. Pliskin, M.A., WAW Division, NIMH; Karen F. Berman, Staff Psychiatrist, CBDB, NIMH; Marvin Podd, Ph.D., Director of Training, Psychology, Bethesda Naval Hospital

COOPERATING UNITS (if any)

Bethesda Naval Hospital

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL MAN-YEARS:

.33

PROFESSIONAL:

.33

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been completed

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02358-03 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Atheoretical Multivariate Statistical Techniques

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Terry Goldberg, Ph.D., Special Expert, CBDB, NIMH

Daniel R. Weinberger, M.D., Chief, CBDB, NIMH, Neil H. Pliskin, M.A., Staff Psychologist, WAW Division, NIMH; Karen F. Berman, M.D., Staff Psychiatrist, CBDB, NIMH; Marvin Podd, Ph.D., Director of Training, Psychology, Bethesda Naval Hospital

COOPERATING UNITS (if any)

Bethesda Naval Hospital

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL MAN-YEARS:

.33

PROFESSIONAL:

.33

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project has been terminated.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02359-02 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Age Disorientation, Mental Status, and Ventricular Brain Ratio

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Terry Goldberg, Ph.D., Special Expert, CBDB, NIMH

Joel E. Kleinman, M.D., Ph.D., Chief, Section on Neuropathology, CBDB, NIMH; Michael S. Myslobodsky, M.D., Visiting Scientist, CBDB, NIMH; David G. Daniel, M.D., Medical Staff Fellow, CBDB, NIMH; Daniel R. Weinberger, M.D., Chief, CBDB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies; Section on Neuropathology

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL MAN-YEARS:

.33

PROFESSIONAL:

.33

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02360-02 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Topographic Analysis of Brain Activity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Richard Coppola, D.Sc., Sen. Engineer, CBDB, NIMH

Terry Goldberg, Ph.D., Special Expert, CBDB, NIMH; Alison Reeve, M.D., Med. Staff Fellow, CBDB, NIMH; Daniel R. Weinberger, M.D., Chief, CBDB, NIMH; Francis Newman, Visiting Scientist, BPB; John M. Morihisa, M.D., Chairman, Dept. of Psychiatry, VAMC; Washington, D.C.; Trey Sunderland, M.D., Med. Officer, LCS, NIMH; Judith M. Rumsey, Ph.D., Sen. Staff Fellow, CPB, NIMH; William H. Theodore, M.D., Research Neurologist, EB/NINCDS; Harold Sackheim, Ph.D., Columbia University, New York; Richard D. Weiner, M.D., Ph.D., VA, Duke University, N.C.; Werner Herrman, M.D., FU, Berlin, W. Germany.

COOPERATING UNITS (if any)

LNP, NIMH; LCM, NIMH; NSB, NIMH; BPB, NIMH; VAMC, Washington, D.C.; LCS, NIAAA; CPB; EB, NINCDS; LNS, NIA; DEB, NICHD; Columbia University, NY; VA, Duke University, N.C.; FU, Berlin, W. Germany

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL MAN-YEARS:

2.5

PROFESSIONAL:

1.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Electrical brain activity, as an index of central nervous system function, is studied across a range of patient groups with neurological and psychiatric disorders as well as normal volunteers. Using electrophysiological data quantified from event-related potentials and spectrum analysis of EEG recordings, computer-derived brain images are able to provide information about neurophysiological function relating to both cognition and clinical state. Topographic maps efficiently characterize spatial and temporal patterns of brain activity allowing the ability to study the dynamic interaction among brain regions and their relation to function.

The project has two main purposes. The first is to refine the topographic and quantitative analysis methods and establish normative data for various conditions and activation procedures. For example, normal subjects differ with respect to their major focus of resting EEG alpha rhythm; one group shows a dominant parietal locus and one an occipital locus, depending on the alpha frequency.

The second purpose is to apply these methods to the characterization of clinical groups and pharmacological response. Work in progress includes characterization of subgroups of Alzheimer's patients, localization of abnormality in epilepsy patients, localization of drug activation and study of psychiatric patients on various neuroleptic drugs..

Project Description:

Objectives: The overall goal of this project is to develop and apply methods for utilizing the electrical activity of the brain as a measure of central nervous system function with the expressed purpose to study human information processing, including attention, sensory processing, and cognition and to study functional states as seen during chronic or transient conditions. Methods have been developed to display topographic maps that efficiently characterize brain activity in terms of both spatial and temporal patterns. The ability to study these patterns in a dynamic fashion will yield a better understanding of the interaction among brain areas and their relation to function.

The project has two main thrusts. The first is to refine the topographic and quantitative analysis methods and establish normative data for the patterns of brain activity seen in a variety of conditions and under various activation procedures. Differentiation of EEG patterns associated with cognitive and attention-related parameters are of importance to understand the underlying neurophysiological basis of both normal and abnormal brain function.

The second thrust is to utilize these methods and normative base to discover and describe characteristic brain activity patterns in a variety of patient groups. The main hypothesis is that certain patient groups will exhibit regional localization of EEG abnormalities. It is expected that quantitative topographic analysis will provide better sensitivity for this localization than the usual clinical EEG recordings. An additional hypothesis is the expectation of changes in quantitative EEG parameters with treatment in patient groups.

Methods Employed: Multilead scalp recordings are made during baseline resting conditions and during various activation procedures. Quantitative reduction of this data is performed by computer spectrum analysis to provide a profile of the energy in the different frequency bands of the EEG. Combining this data with an equal area projection of the scalp surface gives a computer-generated display of a map of brain activity. The map is used to define a baseline condition and changes in the map are used to assess regional patterns during activation procedures. Maps of the raw EEG itself are used to follow the temporal and spatial development of specific EEG events such as a spike and wave complex. Event-related potentials (ERPs) are collected to visual pattern stimulation. Maps are made in a similar fashion for this data and used to determine the intactness of sensory pathways.

Methods employed in specific clinical studies fall into two categories. The first type is where a patient is seen only once. In this case, comparison with other clinical groups or normative data is used to derive characteristic topographic profiles. Correlation or subtyping, using neuropsychological assessment from other studies, may also be carried out. In the second case, patients are seen more than once and assessment is made in regard to change in clinical state, medication, or other treatment.

Collaborative Centers: Because of considerable interest in the research community and as a means to refine these methods and expand the available data base, several collaborating laboratories are now using the system we have developed. This includes laboratories in NINCDS and NIAAA, as well as several outside the NIH.

Major Past Findings: Methodology: We have found that skull asymmetries are correlated with EEG asymmetries thus making it necessary to take structural measures into account when using EEG methods to access laterality.

Previous work had demonstrated that normal subjects fall into two groups with regard to the frequency and spatial location of their alpha rhythm. Those with a peak alpha frequency above 10.2 Hz generally have an occipital pattern and those below 10.2 Hz a parietal pattern. Further studies suggest that these patterns represent stable individual differences even over very short periods of time.

Pharmacological Characteristics: A study of a double blind crossover of placebo, amitriptyline, chlorepromazine, and diazepam has shown specific regional effects in addition to the usual spectrum differences due to these drugs. These data are being used as a model to investigate the complex multivariate statistics needed to analyze these studies. An investigation of nootropic agents has shown that these do not follow a specific EEG profile of changes, but rather depend on characteristics of the baseline EEG and that there are different effects for the same compound with different age subjects.

Epilepsy: Recordings from more than 40 seizure patients have been completed. Some characteristic patterns have emerged from the variety of disorders represented. The dynamic pattern of the spike and wave complex was seen to be almost identical in petit mal patients as well as other spike-wave. In all cases, the spike has a mid-line frontal maximum that does not shift position during the time course of the spike itself.

A comparison on quantitative EEG to other neuro-imaging methods has shown good agreement, even in several cases when routine surface EEG was equivocal.

Clinical Studies: Dementia: Increased slow wave activity and decreased alpha amplitude differentiate Alzheimer patients from both elderly depressed patients and normals. The amount of increased delta activity correlates with a variety of clinical and behavioral measures in the Alzheimer group.

Schizophrenia: Low alpha frequency occurred more commonly in schizophrenic patients than in normal controls. Those patients with low alpha frequency had larger ventricles than did other patients. Alpha frequency was inversely correlated with the ventricular brain ratio.

Significance to Biomedical Research and to the Program of the Institute: Electrical activity recorded at the scalp is currently the only non-invasive technique available as a window on the physiological functioning of the human brain. While EEG is a very indirect measure of neural activity, its advantage is the ability to reflect changes on a millisecond-by-millisecond basis. This allows EEG to be related to behavior in an ongoing manner. In contrast, PET images have higher resolution and more directly measure neural activity but reflect the summation of activity over a period of correlation of activity with behavior. EEG imaging gives complementary data to the other modalities of cerebral metabolism (PET) and blood flow (rCBF).

Publications:

Coppola R, Herrman WM: Psychotropic drug profiles: Comparisons by topographic maps of absolute power. Neuropsychobiology 18:97-104, 1987.

Coppola R, Morgan NT: Multi-channel amplifier system for computerized topographic EEG analysis. Electroencephalogr Clin Neurophysiol 67:191-193, 1987.

Coppola R, Karson C, Daniel D, Myslobodsky M: EEG asymmetry in relation to skull asymmetry. J Clin Neuropsychol 4:282, 1987.

Niederhoffer RG, Gabrielli JDE, Coppola R: Topographic electroencephalograph correlates of the perception of rhythm. Soc Neurosci Abstr 13:849, 1987.

Kahn EM, Weiner RD, Brenner RP, Coppola R: Topographic maps of brain electrical activity - technical consideration. Biol Psychiatry 23:628-636, 1988.

Karson C, Coppola R, Daniel D: Alpha frequency in schizophrenia: An association with enlarged

cerebral ventricles. Am J Psychiatry, in press.

Coppola R: Topographic display of spike-wave discharges. In Myslobodsky M and Mirsky A (Eds): Petit Mal Epilepsy: Basic Mechanisms. Peter Lang, New York, in press.

Coppola R: Interpretation of multilead data. In Rohrbaugh JW, Johnson R and Parasuraman R (Eds): Event-related Potentials of the Brain. Oxford, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02388-03 CRDR

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regional Cerebral Blood Flow in Neuropsychiatric Patients and in Normal Subjects

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Terry Goldberg, Ph.D., Special Expert, CBDB, NIMH

Daniel R. Weinberger, M.D., Chief, CBDB, NIMH, Neil H. Pliskin, M.A., Staff Psychologist, WAW Division, NIMH; Karen F. Berman, M.D., Staff Psychiatrist, CBDB, NIMH; Marvin Podd, Ph.D., Director of Training, Psychology, Bethesda Naval Hospital

COOPERATING UNITS (if any)

Bethesda Naval Hospital

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL MAN-YEARS:

.33

PROFESSIONAL:

.33

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been completed.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02389-03 CBDE

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Electrical Activity Mapping in Neuropsychiatric Patients

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Craig N. Karson, M.D., Staff Psychiatrist, CBDB, NIMH

Terry Goldberg, Ph.D., Special Expert, CBDB, NIMH; Karen F. Berman, M.D., Staff Psychiatrist, CBDB, NIMH; Ralph Fawcett, M.D., Medical Staff Fellow, NPB, NIMH; Richard Coppola, D.Sc., Senior Engineer, CBDB, NIMH; Daniel R. Weinberger, M.D., Chief, CBDB, NIMH

COOPERATING UNITS (if any)

Neuropsychiatry Branch, NIMH

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL MAN-YEARS:

2

PROFESSIONAL:

.5

OTHER:

.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

This project has been terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02390-02 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

An Exploration of Parietal Functions in Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Michael Myslobodsky, M.D., CBDB, NIMH

Terry Goldberg, Ph.D., Special Expert, CBDB, NIMH; Daniel R. Weinberger, M.D., Chief, CBDB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL MAN-YEARS:

.1

PROFESSIONAL:

3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02391-03 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Phenomena In Schizophrenia and the Development of Novel Treatments

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Craig N. Karson, M.D., Staff Psychiatrist, CBDB, NIMH

David G. Daniel, M.D., Medical Staff Fellow, CBDB, NIMH; Llewellyn B. Bigelow, M.D., Senior Staff Fellow, CBDB, NIMH; Darrell G. Kirch, M.D., Clinical Director NRH, NIMH; Joel E. Kleinman, M.D., Ph.D., Chief, Section on Neuropathology, CBDB, NIMH

COOPERATING UNITS (if any)

Neuropsychiatric Research Hospital

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies; Section on Neuropathology

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL MAN-YEARS:

.6

PROFESSIONAL:

.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02392-02 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluation Of Patients With Prefrontal Leukotomies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Karen Faith Berman, M.D., Staff Psychiatrist, Clinical Brain Disorders Branch, IRP, NIMH; Barbara P. Illowsky, M.D., Medical Staff Fellow, Clinical Brain Disorders Branch IRP, NIMH; Daniel R. Weinberger, M.D., Chief, Clinical brain Disorders Branch

Denise Juliano, MSW, Social Worker, Clinical Services Branch, WAW Division
Richard Suddath, M.D., Medical Staff Fellow, NPB, DIRP, NIMH; Terry E. Goldberg, Ph.D., Special Expert, CBDB, IRP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section On Neuropathology Studies

INSTITUTE AND LOCATION

NIMH, Neuroscience Center at Saint Elizabeths

TOTAL MAN-YEARS:

11

PROFESSIONAL:

6

OTHER:

2

CHECK APPROPRIATE BOXES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Prefrontal lobotomies for psychiatric illness were last performed in the 1950's. Approximately 40 patients who had dorsolateral prefrontal lobotomies remain on the patient rolls at St. Elizabeths Hospital. The long-term effects of prefrontal lobotomy and the overall outcome of the patients is not clearly known. Techniques are now available to study anatomic, clinical and physiological aspects of lobotomy patients. These include computerized tomography scanning (CT), magnetic resonance imaging (MRI), and cerebral blood flow (cbf) examination. Neurological, psychiatric, and psychological evaluation of these patients in correlation with the above examinations compared to age and diagnosis matched-non-lobotomized patients, will give information on both the effects of prefrontal lobotomy and on the function of prefrontal cortex.

Project Description:

Objectives: Detailed physiological, clinical, and anatomic study of patients 30-40 years after prefrontal leukotomy have not been done. This study will add to the fund of knowledge on the long-term effects of lobotomy, and, more generally, contribute to understanding frontal lobe function.

Methods Employed: Patients who underwent prefrontal lobotomies have been identified either from St. Elizabeths records or from recognition of typical lesions on CT (computerized tomography) scans obtained for other reasons. Controls are being selected from the St. Elizabeths roster and matched for age, diagnosis, and duration of hospitalization. The patients receive a neurological examination and neuropsychological battery. CT and MRI scans are used to define the lesions. Cerebral blood flow evaluation using Xenon 133 inhalation during the performance of cognitive tasks provides a measure of physiological function.

Major Findings: The study is still in progress to date, we have finished neurologic examination of 19 lobotomy patients and 16 controls.

Neuropsychological testing is complete on 11 patients, CT scans on 18, MRI scans on 11 and rCBF on 4. Work is continuing on examination of more patients and controls, measurements of scans and inter-correlation of the data obtained.

Proposed Course Of Project: The project will run until all known lobotomy patients who are able to participate have been examined.

Papers: Illowsky BP, Berman KF, Daniel DG, Sudath R, Juliano DM, Weinberger Dr. CT and MRI scans in prefrontal Leubotomy presented at the Society for Biological Psychiatry meeting, Montreal, Canada, May 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02393-02 CBDB
PERIOD COVERED October 1, 1987 through September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Demeclocycline In The Treatment Of Psychogenic Polydipsia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Barbara P. Illowsky, M.D., Medical Staff Fellow, Clinical Brain Disorders Branch, NIMH George Christison, M.D., Staff Fellow, Clinical Brain Disorders Branch, NIMH; Darrell G. Kirch, M.D., Senior Staff Fellow, NPB,DIRP,NIMH;		
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Brain Disorders Branch		
SECTION Section On Neuropathology Studies		
INSTITUTE AND LOCATION NIMH, Neuroscience Center at Saint Elizabeths		
TOTAL MAN-YEARS: <div style="text-align: center; margin-top: 5px;">6</div>	PROFESSIONAL: <div style="text-align: center; margin-top: 5px;">3</div>	OTHER: <div style="text-align: center; margin-top: 5px;">1</div>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Patients with <u>psychogenic polydipsia</u> (compulsive water drinking) comprise about 6% of the schizophrenic population. These patients are usually managed by imposed fluid restriction with symptomatic treatment for episodic water intoxication. Many of these patients have been shown to have inappropriate secretion of <u>antidiuretic hormone</u> (SIADH) which may be a factor in their episodes of hyponatremia.</p> <p><u>Demeclocycline</u> is an antibiotic related to tetracycline which blocks the action of antidiuretic hormone (ADH) and is useful in treating SIADH of diverse etiologies. Preliminary study has suggested that demeclocycline might be useful in psychiatric patients with psychogenic polydipsia.</p>		

Project Description:

Objectives: This study is designed to evaluate the efficacy of demeclocycline in maintaining normal sodium balance in patients with psychogenic polydipsia and in preventing water intoxication.

Methods Employed: Patients are selected for participation in the study based on past history of compulsive water drinking and water intoxication. They frequently have mild chronic hyponatremia and hyposthenuria. The study incorporates a double-blind, placebo-controlled, cross-over design. The patients receive either three weeks of placebo or three weeks of demeclocycline in gradually increasing dosage. During the study, patients are examined for changes in drinking behavior, weight gain (which may indicate an abrupt increase in fluid intake) and psychosis. Serum sodium level and osmolarity are monitored. If symptomatic or severe hyponatremia develops while in the study, fluid restriction of undertaken to ensure the safety of the patient.

Major Findings: Six trials have been completed to date. The preliminary results indicate that demeclocycline is useful in modulating wide fluctuations in serum sodium level and in decreasing episodes of water intoxication which require fluid restriction.

Significance To Mental Health Research: If demeclocycline proves efficacious in decreasing episodes of symptomatic water intoxication, psychiatrists will have another method for treating this subgroup of patients. Fluid restriction requires close nursing supervision and entails a great degree of discomfort for the patient. Demeclocycline would be easier for both the staff and the patient to tolerate and may raise the level of compliance with treatment.

Proposed Course Of Project: The project will run until 10-15 trials have been completed.

Publications:

Illowsky BP, Kirch DG: Polydipsia and hyponatremia in psychiatric patients. Am. J. Psychiatry 145:675-683, 1988.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02394-03 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Magnetic Resonance Imaging (MRI) Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Daniel R. Weinberger, M.D., Chief, CBDB, NIMH

Richard Suddath, M.D., Medical Staff Fellow, NPB, NIMH; John Kelsoe, M.D., Medical Staff Fellow, Clinical, CNB, NIMH; David Pickar, M.D., Chief, Section on Clinical Studies, CNB, NIMH; Manual Casanova, M.D., CBDB, George Christison, M.D., Terry Goldberg, Ph.D., Fuller Torrey, M.D.

COOPERATING UNITS (if any)

Section on Clinical Studies, CNB, NIMH

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL MAN-YEARS:

3

PROFESSIONAL:

1

OTHER:

1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are studying the neuroanatomical localization of the possible underlying pathology using MRI. Attempts will be made to correlate neuroanatomical abnormalities to other findings such as those seen in blood flow or BEAM studies. These studies are using state of the arts digitized image analysis system to examine gray-white matter quantitative differences between schizophrenic and normal. Preliminary results indicate that patients have larger ventricles and less temporal gray matter. This represents potentially important evidence of a relatively focal pathological process.

Projective Description:

Objectives: We are continuing to study the neuroanatomy of the brains of schizophrenics by using the more sophisticated non-radiologic technique, MRI, which might allow better delineation of the abnormalities that have been reported using the CT scan.

Methods Employed: In vivo MRI focuses on signals retrievable from hydrogen 1. The images obtained provide better delineation of brain anatomy than the CT scan. With MRI, there is a greater gray-white matter contrast and the images in various brain areas that have previously been difficult to evaluate. Furthermore, as the meaning of signal intensity becomes better understood, we will be able to draw conclusions about the degree of pathology according to differences in MRI signal intensities.

Over 50 patients and normal controls have been scanned with the NIH Clinical Center machine (.5 tesla). Data have been collected and evaluated by use of a computerized system that allows area measurements on these scans. Size of ventricles, cortical areas, basal ganglia anatomy, and the anatomy of some of the forebrain nuclei are among the structures that can be evaluated using this system. The results of the first study have been submitted for publication. Total ventricular volume was significantly enlarged in the patients. In a follow-up study using a refined computerized image analysis system, we have found reduced volume of temporal gray matter.

In addition we have begun to examine discordant monozygotic twins using a more powerful 1.5 T machine that provides improved gray-white matter resolution. We have currently examined over five pairs of twins.

Significance to Mental Health Research: This study may help to determine the basic neuroanatomical lesions(s) which caused the abnormalities observed in the brains of schizophrenics, i.e., whether the pathology is related to cortical or subcortical lesions, is local diffuse.

Proposed Course of Project: This study will continue during the next few years and it is hoped that it will be able to provide better data as to the basic pathology involved in the causation of the abnormalities seen on CT scans in the brains of schizophrenics, and to correlate these data with clinical variables.

Publications:

Kelsoe J, Cadet J-L, Pickar D, Weinberger DR: Quantitative neuroanatomy in schizophrenia: A controlled MRI study. Arch Gen Psychiatry 45:533-541, 1988.

Suddath RL, Casanova M, Goldberg T, Daniel D, Kelsoe J, Weinberger DR: Temporal lobe pathology in schizophrenia: A quantitative MRI study. Am J. Psychiatry (in press).

Berman KF, Weinberger DR: Brain structure and function in schizophrenia. In: Comprehensive Textbook of psychiatry, 5th ed, Kaplan HI, Sadock BJ (eds). Williams & Wilkins, 1989 (in press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02395-03 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Brain Imaging In Schizophrenic Patients And Normal Subjects

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Daniel R. Weinberger, M.D., Chief, CBDB, Branch, NPB, IRP, NIMH

David G. Daniel, M.D., CBDB; George Jaskiw, M.D., Visiting Associate, CBDB, NIMH; Barbara Illowsky, M.D., Medical Staff Fellow, CBDB, NIMH

COOPERATING UNITS (If any)

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL MAN-YEARS:

2

PROFESSIONAL:

1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The projects on structural brain imaging investigates structural pathology of the brains of schizophrenic patients housed in the William A. White research units using x-ray computerized tomography (CT). Patients are compared to matched normal controls. The most recent study, a culmination of four years of data collection, compared 73 schizophrenic patients to 30 normal volunteer controls. This project is a replication and extension of the previous work done in this area in the branch. Using standardized techniques four brain areas were examined: Lateral ventricles, third ventricles, cortical (parieto-occipital) areas, and prefrontal cortex. In this sample, the lateral and third ventricles continued to be significantly larger in patients than controls. A potentially exciting new finding was that though there were essentially no differences between patients and controls in cortical atrophy in the parieto occipital distribution, the schizophrenic patients showed substantially greater atrophy in the prefrontal distribution, localizing the cortical changes to this area. Further, in a subgroup of 18 drug-free and 22 medicated patients, the CT abnormalities were correlated with regional cerebral blood flow (rCBF) using the radioactive ¹³³Xenon inhalation technique. Relationships were found between the neurophysiological measurements and CT scanning, especially in the prefrontal cortex and ventricular areas. This work is being amplified to search for clinical and biological correlations of ventricular enlargement and prefrontal atrophy, particularly with respect to other signs of prefrontal pathology, e.g. rCBF, EEG, PET data. In addition, we followed up earlier patients, rescanning them after 9-9 years. We have found no change over time, indicating that the pathology underlying these changes probably static.

Project Description:

Objectives: Before the advent of CT scanning, observations of the human brain were largely indirect: chemical markers of brain metabolic activity in the cerebrospinal fluid, blood, or urine; responses to centrally-acting medications; animal models of human brain function; post-mortem brain studies; and crude methods of visualizing brain structures such as pneumoencephalography. CT scanning proved a major advance in providing detailed pictures of cross-sections of brain in living subjects with minimal risk. These accurate and reliable methods could then be applied to the study of brain pathology in such diseases as schizophrenia.

We have used CT observations to study a number of parameters of brain structural abnormalities in schizophrenic patients. Reversed cerebral asymmetries and cerebellar atrophy have been described in patients, but the first and most venerable finds were those implicating atrophy of the cerebrum. In particular, previous studies have indicated enlargement of lateral and third ventricles and atrophy of the cortical surface, finds apparently unrelated to age, neuroleptic exposure or duration of illness, but adjustment, diminished response to neuroleptics, poorer outcome, cognitive impairment, and other negative symptoms. Additionally, these changes are apparently present at the onset of illness.

Over the last year the laboratory has tried to meet several objectives: (i) To replicate the previous work showing cerebral atrophy in a sample of schizophrenic patients more representative of the broad distribution of affected persons. (ii) to extend the work by attempting to localize the site of cortical atrophy and (iii) to relate the changes on CT scan to physiological measures by rCBF, especially so-called "hypofrontality" (i.e., relatively diminished prefrontal blood flows under conditions of cognitive stimulation of this area).

To achieve these results, CT and rCBF data were collected along with clinical information and neuropsychological testing.

Methods Employed: Patients selected for study were housed in the clinical research units, Saint Elizabeths Hospital, William A. White Building, and were rigorously diagnosed as having schizophrenia by DSM-III criteria. Normal volunteers were obtained via several investigators at the NIH Clinical Center, Bethesda, Maryland. All subjects underwent standard CT scanning with the same GE 8800 scanner at the Clinical Center. Twelve to 13 images or slices were produced at 15° to the cantho-meatal line. Measurements were made from these images on photographic film.

Lateral ventricle size was measured using a fixed-arm planimeter, an engineering device used to measure the area of irregular two-dimensional structures. The ventricular area is divided by the area of the whole brain, multiplying by 100, giving a percentage size or ventricular-brain ratio (VBR). Third ventricular size is measured by laying a mm ruler across the greatest diameter, then multiplying by a so-called "magnification factor" of 2.7 (the relationship between the photographic image and the true size of the subject's brain). Generalized (parieto-occipital) atrophy was evaluated on an appropriate slice with a 0 (mild) to 3 (severe) scale with half-steps between (e.g., 0.5, 1.5) by referring to standard examples. Prefrontal atrophy was similarly evaluated on a scale derived from CT cuts at an appropriate position. The patient data derived were compared to the same measurements from volunteer controls. All measurements were performed blind to patients vs controls. A subsample of patients were selected to compare CT changes and abnormalities on rCBF described in detail under another heading.

Major Past Findings: In a relatively severely-impaired sample of patients, there was evidence of abnormal enlargement of lateral and third ventricles and cortical atrophy (measured with a different scale). These abnormalities did not relate to clinical parameters such as age, duration of illness or hospitalization or neuroleptic treatment, but were correlated of illness or premorbid adjustment, cognitive impairment, poor response to neuroleptics and poor outcome. Other abnormalities discovered included an increased incidence of cerebellar atrophy and reversed cerebral asymmetries in the patients. Additionally, evidence of prefrontal atrophy was correlated with increased electrical activity mapping (BEAM), a computerized evaluation of the electroencephalogram.

New Findings: Comparing the 73 schizophrenic patients to 30 normal volunteer controls, enlargement of lateral and third ventricles was again in this somewhat less-severely impaired patient sample, though still reaching statistical significance. There were no differences between patients and controls on the generalized (parieto-occipital) scale, but significant differences in the prefrontal distribution indicating a localization of atrophy in this area. This is consistent with some findings from rCBF, BEAM, Positron Emission Tomographic Scanning and Neuropsychological deficits found in schizophrenic patients. This is also significant in light of the similarities of symptoms found in person with known injuries of the dorsolateral prefrontal cortex and the so-called "core" or "defect" symptoms of schizophrenia including flattened affect, social impairment, apathy, withdrawal, etc.

Abnormalities on rCBF, in particular the so called hypofrontality seen in schizophrenic patients under conditions of specific neuropsychological stimulation (the Wisconsin Card Sort test, and activator of the dorsolateral drug-free and 22 medicated patients. This seems to indicate relationships between physiological dysfunction in the prefrontal cortex and structural abnormalities in both prefrontal and subcortical areas. This consistent with a developing knowledge base from basic research indicating strong structures that may be abnormal in schizophrenia. Finally, we have rescanned 20 patients after 7-9 years and find no evidence of progression of their structural pathological condition.

Significance to Mental Health Research: Understanding the basic structural and physiological dysfunction in the brains of schizophrenic patients is vital to the progress of research in the illness. Such underpinning will allow the development of specific neurorehabilitative paradigms and potentially an understanding of the etiologies of the illness. By comparing structural and functional measurements, abnormalities can be better localized in the brain, and can be related to specific clinical parameters. The primary goal, then, is to clarify the site of the "critical lesions" in the brains schizophrenic patients, i.e., those areas primarily effected in the illness, accounting for the core symptoms.

Proposed Course of Project: With the extensive CT data collected, correlations will be made with various clinical and psychological parameters, e.g., cognitive impairment, "negative" and "positive" symptoms and neuroleptic responsiveness. We also are utilizing sophisticated computerized image analysis system to analyze the vast amount of data contained in CT image. Plans are also being formulated to utilize an exciting new imaging technique, nuclear magnetic resonance (NMR). This technique will allow structural imaging in exquisite detail, revealing more specifically areas such as individual periventricular nuclei, depth of cortical and periventricular gray matter, and giving a much more specific "look" at brain structural abnormalities.

Publication:

Lawson WB, Waldman IN and Weinberger DR: Schizophrenic dementia: clinical and CT correlates. J Nerv Ment Dis 176:207-212, 1988.

Goldberg TE, Kleinman JE, Daniel DG, Myslobodsky MS, Ragland JR, Weinberger DR: Dementia praecox revisited: Age disorientation, mental status, and ventricular enlargement. Br J Psychiatry 153:187-190, 1988.

Casanova MF, Prasad CM, Karp B, Stein B, Weinberger DR, Kleinman JE: Basal ganglia mineralization in chronic psychiatric patients. Biol Psychiatry (in press).

Myslobodsky MS and Weinberg DR: Reversed brain anatomical asymmetries in schizophrenia: A search for contributing variables. In: Duality and Unity of the Brain, Ottoson D (ed). MacMillan Press, London, 1987, pp. 367-387.

Weinberger DR: A neurodevelopmental perspective a brain pathology in schizophrenia. In: Etio-pathogenic Hypotheses of Schizophrenia, Sachtell EM (ed). MTP Press, London, 1987, pp. 59-65.

Weinberger DR: Schizophrenia and the frontal lobes. Trends Neurosci 11:367-370, 1988.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02397-03 CBDB

PERIOD COVERED

October, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hierarchy and Sensitivity in Putative Frontal Lobe Tasks

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Terry Goldberg, Ph.D., Special Expert, CBDB, NIMH

Daniel R. Weinberger, M.D., Chief, CBDB, NIMH; John Kelsoe, M.D., CNB, NIMH

COOPERATING UNITS (if any)

Section on Clinical Studies, CNB

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL MAN-YEARS:

.33

PROFESSIONAL:

.33

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

This project has been terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02398-02 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of an Auditory Sort Test

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Terry Goldberg, Ph.D., Special Expert, CBDB, NIMH

Daniel Weinberger, M.D., Chief, CBDB, NIMH; Craig Karson, M.D., Staff Psychiatrist, CBDB, NIMH; Karen F. Berman, M.D., Staff Psychiatrist, CBDB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL MAN-YEARS:

.35

PROFESSIONAL:

.35

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Concepts formation tasks with a set shifting component have proven sensitive to frontal lobe dysfunction. The Wisconsin Card Sort, perhaps the most widely known test of this class, activates prefrontal regions in most normal but no schizophrenic subjects. It is presented in the visual modality. To further validate the use of such a test and to facilitate cognitive activation in EEG-BEAM studies while reducing eye movement, an auditory analog of the Card Sort was developed.

Project Description:

Objectives: The Auditory Sort Test is a non-verbal auditory task. It has two functions. The first is to differentially activate brain regions, namely temporal and prefrontal. The second is to display adequate psychometric properties (reliability, concurrent validity with Wisconsin Card Sort, independence from motivation and attentional factors, and separation of groups).

Methods Employed: Tones are presented to the subject and she/he is asked to match each to one of two target tones. Tones may be matched on the basis of tone quality or duration. After the subject makes 10 successive correct matches, the categorization shifts without warning. There are 60 items in the test and it takes about 10 minutes to administer. Normal and schizophrenic subjects are administered the test along with the Wisconsin Card Sort and the Auditory Continuous Performance Task (of vigilance and attention). Tones are created by a speech synthesizer (DecTalk).

Major Findings: This study is in progress.

Significance to Mental Health Research: The Auditory Sort Test may prove a significant tool in activating specific regions of the brain. Moreover, performance among different groups (schizophrenic, neurologic, normal) may be a sensitive indicator of dysfunction.

Proposed Course of Project: The project will continue until a data collection is complete. Patients undergoing both cerebral blood flow and computerized EEG will receive the test (in a time locked format for the latter). Further studies on neurologic patients with discrete regions and non-schizophrenic psychiatric are planned.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02399-03 CBDB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Postmortem Brain Tissue Examination in Neuropsychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Joel E. Kleinman, M.D., Ph.D., Deputy Chief, CBDB, IRP, NIMH

Manuel F. Casanova, M.D., Neuropathologist, CBDB; George Jaskiw, M.D., Staff Psychiatrist CBDB, William J. Freed, Ph.D., Section Chief, NPB, IRP, NIMH; Markku Linnoila, M.D., Ph.D., Chief, LCS, NIAAA; Michael J. Kuhar, Ph.D., Chief Neuroscience Branch, NIDA

COOPERATING UNITS (if any)

NPB, NIMH, NCB, NIDA, LCS, NIAAA

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, Neuroscience Center at Saint Elizabeth's

TOTAL MAN-YEARS:

5

PROFESSIONAL:

3

OTHER:

2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Postmortem Studies in neuropsychiatric disorders test hypotheses with regard to schizophrenia, suicide, and addictions. New findings in schizophrenia include the following: (1) increased met-enkephalin in the substantia nigra of schizophrenic patients versus controls; (2) normal dopamine reuptake sites in putamen of schizophrenic patients versus controls; (3) an association of age disorientation (an aspect of dementia) with ventriculomegaly in schizophrenic patients; and (4) no evidence for hippocampal disarray in schizophrenic patients.

New findings in suicide studies included normal amino acid concentrations in a number of brain regions. In addition, a study of heat shock proteins found that heterozygosity was present in 30% of brains of subjects who died from causes other than suicide. However, in 21 suicides (100%), the heat shock proteins were homozygous.

Lastly, hippocampal glutamate binding was increased in alcoholic brains as compared with controls. This increase was especially pronounced in alcoholics who died from seizures secondary to alcohol withdrawal.

Project Description:

Objectives: Neurochemical analyses of postmortem human brain tissue is an area of increasing interest to psychiatric research. Many groups are now concentrating on mapping central neuronal pathways or identifying biochemical abnormalities in neurological and psychiatric diseases. Our studies have focused primarily on neurochemical hypotheses in schizophrenia and suicide with some work on aging, Parkinson's disease and addiction. In the schizophrenic syndrome our efforts have focused mostly on catecholamines, (especially receptors), indoleamines, neuropeptides and amino acids. Viral hypotheses have also been tested using schizophrenic brain specimens. In suicide studies, the emphasis has been on indoleamines, norepinephrine, acetylcholine, amino acids, and proteins. In aging, studies have examined dopamine receptors (types 1 and 2) and dopamine reuptake mechanisms. The latter has been the focus of Parkinson's disease studies in the past. Addiction studies have focused on PCP and opiate binding sites, while studies in alcoholism have involved glutamate receptors.

Methods Employed: Brains are collected seven days a week from the D.C. Medical Examiners office by the neuropathologist and his assistants. Patient and control brains are dissected by the neuropathologist and a research assistant according to international criteria. Careful matching for postmortem interval and for freezer storage time is done in addition to the routine age, race, and gender matching. Another variable that may require matching is the time of year at death. Diagnosis of patients is performed independently by two psychiatrists, using the newly developed Diagnostic Evaluation After Death criteria.

New Findings:

Schizophrenia - (1) increased substantia nigra met-enkephalin concentrations relative to controls; (2) Normal putamen dopamine reuptake sites relative to controls; (3) no hippocampal disarray relative to controls; (4) an association of age disorientation (a measure of dementia) with ventriculomegaly; and (5) no evidence for viral transmission from schizophrenic brain tissue.

Suicide Studies; (1) Normal concentrations of amino acids in a number of brain regions relative to controls; and (2) evidence for homozygosity of heat shock proteins in suicides in contrast to normals where some specimens demonstrate heterozygosity.

Alcoholism: Increased hippocampal glutamate binding relative to controls especially in alcohol withdrawal seizures.

Significance to mental Health Research: These studies are designed to elucidate neurochemical and structural abnormalities in the brains of schizophrenics, suicides, alcoholics, and heroin addicts. This understanding will hopefully lead to new treatment and prevention.

Proposed Course of Project: Schizophrenia (1) Attempts to replicate and examine dopamine receptors in other brain regions are currently underway; (2) another project in progress involves measurements of catecholamines in a number of limbic nuclei; (3) attempts to measure glutamate receptors are underway; (4) new methods to study brains with neuronal morphometrics, immuno-cytochemistry, autoradiography and in situ DNA hybridization are in progress or are planned.

Suicides and Alcoholism: Studies looking at heat shock proteins are in progress. Further studies looking at indoleamines and metabolites are planned for better diagnosed patients.

Heroin Addiction: Further work on opiate receptors and endogenous opiate compounds are planned.

Publications:

Kaufmann, C.A., Weinberger, D.R., Stevens, J.R., Asher, D.M., Kleinman, J.E., Sulima, M.R., Gibbs, C.J. and Gajdusek, D.C.: Intracerebral inoculation of experimental animals with brain tissue from patients with schizophrenia. Failure to observe behavioral and neuropathological effects. Arch. of Gen. Psychiatry 45:648-652, 1988.

Jaskiw, G. and Kleinman, J.E.: Postmortem neurochemistry studies in schizophrenia. In: Schulz, S.C. and Tamminga, C.A. (eds.): Schizophrenia: A scientific focus. New York, Oxford University Press (in press).

Korpi, E.R., Kleinman, J.E. and Wyatt, R.J.: GABA Concentrations in forebrain areas of suicide victims. Biol. Psychiatry 23: 109-114, 1988.

Goldberg, T., Kleinman, J.E. and Weinberger, D.R.: Dementia praecox revisited, Br. J. Psychiatry (in press).

Kleinman, J.E., Casanova, M.F. and Jaskiw, G.E.: The neuropathology of schizophrenia. Schizophrenia Bulletin (in press).

Wagner H.N., Kleinman, J.E., Weinberger, D.R., Casanova, M.F., Coppola, R., Gibbs, C.J., Gur, R.E., Hornykiewicz, O., Kuhar, M.J., Pettegrew, J.W. and Seeman P. Report of the panel on neuroimaging and neuropathology Schiz. Bull. 14 (in press).

Christison, G.W., Casanova, M.F., Weinberger, D.R., Rawlings, R. and Kleinman, J.E.: A quantitative investigation of hippocampal cell size, shape and variability of orientation in schizophrenia. Arch. of Gen. Psychiatry (in press).

Iadarola, M.J. Ofri, D. and Kleinman, J.E.: Enkephalin, dynorphin and substance P in postmortem substantia nigra from normals and schizophrenic patients. Life Sciences (submitted).

Michaelis, E.K., Galton, N., Kleinman J.E. and Freed, W.J.: Glutamate receptor changes in brain synaptic membranes from human alcoholics Science (submitted).

Sharkey, J. Milberger, M., Casanova, M.F., Kleinman, J.E., and Kuhar, M.J.: Dopamine uptake sites in human putamen labeled by [3H]-GBR 12935: Lack of change in schizophrenia. Neuropsychopharmacology (submitted).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02400-0 2 CBDB
PERIOD COVERED October 1, 1987 through September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Eight Year Follow-up of Ventricular Size In Schizophrenia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Barbara P. Illowsky, M.D., Medical Staff Fellow, Clinical Brain Disorders Branch, NPB, IRP, NIMH Denise M. Juliano, MSW, Social Worker, Clinical Services Branch, WAW Division; Dr. Llewellyn B. Bigelow, Associate Clinical Director, WAW Division, Saint Elizabeths Hospital, NIMH; Daniel R. Weinberger, M.D., Chief, Clinical Brain Disorders Branch, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Brain Disorders Branch		
SECTION Section On Neuropathology Studies		
INSTITUTE AND LOCATION NIMH, Neuroscience Center at Saint Elizabeths		
TOTAL MAN-YEARS: 2	PROFESSIONAL: 3	OTHER: 2
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) This project has been completed. Full reference citation is: JNNP 51(2): 209-213, 1988.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02401-01 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropsychology of Twins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

E. Fuller Torrey, M.D.

Terry Goldberg, Ph.D., Special Expert, CBDB, NIMH

COOPERATING UNITS (if any)

Twin Studies Unit

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL MAN-YEARS:

.33

PROFESSIONAL:

.33

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The neuropsychological study of twins with psychiatric disorders has rarely been attempted. In the present study, groups of monozygotic twins whose members are both normal, whose members are both schizophrenic, and whose members are discordant for schizophrenia (i.e., one member is normal and one member is schizophrenic) will be assessed with a complete neuropsychological battery. Included tasks that assess motor functioning, attentional skills, memory abilities, higher level abstract problem solving, and basic language and visual spatial functions. Also tests of dichotic listening with both neutral and affectively charged words were administered to assess laterality and emotional responsivity. By the use of matched pair design in which both genetic and early social environment is more or less completely controlled, as well as the experience of twinship itself, it is hoped that the pattern of deficits found will elucidate impairment in schizophrenic disease.

Project Description:

Objectives: It is hypothesized that schizophrenic patients in this study will exhibit differential deficits in tests of higher level problem solving, recall, and working memory that may reflect the integrity of prefrontal cortex. In addition, milder deficits in attentional functioning, and basic linguistic and visual spatial functioning will also be noted.

Methods Employed: A neuropsychological battery of approximately three hours is administered to each subject. Performance on a state trait measure of anxiety is used to control for subjects response to the testing situation. In addition, a select number of tests will be administered again to control for anxiety in the testing situation.

Major Past Findings: The study is in progress.

Significance to Mental Health Research: The study may provide findings that bear upon the central deficits in the cognition of schizophrenia that cannot be attributed to genetic or social environment. It might provide a focus for strategies to ameliorate them. Furthermore, the results will be correlated with measures that range from quantified MRIs, detailed personality and symptomatological interview data, and metabolic studies.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02434-01 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropsychological Test Findings and MRIs: A Correlative Study

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Terry Goldberg, Ph.D., Special Expert, CBDB, NIMH

Richard Suddath, M.D., Sen. Staff Fellow, NPB, NIMH; Manuel F. Casanova, M.D., Medical Officer, CBDB, NIMH; Daniel R. Weinberger, M.D., Chief, CBDB, NIMH

COOPERATING UNITS (if any)

Neuropsychiatry Branch, NIMH

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies; Clinical Neuropsychiatry, Neuropathology

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL MAN-YEARS:

.15

PROFESSIONAL:

.15

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Computerized image analysis of MRIs has proved to be a valid method of assessing volume in vivo of selected brain structures. The use of one such system (LOATS) enables one to determine the size of such structures as frontal lobe, temporal lobe, caudate, thalamus, and corpus callosum in various coronal and sagittal plains through the use of an edge finding function. In addition, gray matter can be distinguished from white matter and the ventricular system size can be delineated. These measurements can, in turn, be correlated with performance on various neuropsychological measures including Verbal IQ, Performance IQ, Wechsler Memory Scale MQ and Halstead-Reitan average impairment rating. Volumetric reductions in specific brain areas may, in fact, be associated with focal neuropsychological deficits.

Project Description:

Objectives: Improvements in the specificity and selectivity of neuropsychological assessment procedures and in high technology image analysis allow for the possibility that abnormalities in specific neuropsychological tests may be associated with specific abnormalities (either in volume or in shape) in a brain area.

Methods Employed: MRI image analysis will be made on the LOATS system. The size of various structures, as well as their shape, will be quantified. These data will, in turn, be correlated with performance on various neuropsychological tests routinely administered to patients in the program.

Major Past Findings: The study is in progress

Significance to Mental Health Research: The study may provide valuable links between neuropathology and cognitive impairment, which, in turn, may be responsible for basic adaptive deficits that occur in patients with schizophrenia. Such findings would strengthen the causal chain of schizophrenic breakdown and would help to concentrate research efforts in specific brain areas and neuropsychological functions.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02435-01 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Performance of Chronic Schizophrenic Patients on the Chapman Scales

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Terry Goldberg, Ph.D.; Special Expert, CBDB, NIMH

Jlm Gold, Ph.D., Psychologist; Daniel R. Weinberger, M.D., Chief, CBDB, NIMH

COOPERATING UNITS (if any)

Clinical Services Branch

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies; Clinical Services

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL MAN-YEARS:

.10

PROFESSIONAL:

.10

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Ways to measure psychopathology in schizophrenia are numerous. However, the relation of these symptoms to other variables of interest (namely, brain images and neuropsychological performances) has been somewhat disappointing. Therefore, it is important to search for an inventory of symptoms, attitudes, beliefs, and feelings that might, in fact, be linked to neuropsychological deficit and neuroanatomic or neurophysiologic abnormality. The Chapman/Scales of social anhedonia, physical anhedonia, perceptual aberration, magical ideation, and non-conformity have been carefully structured and assess symptoms often associated with defect states and symptoms associated with florid psychotic states. The scales were designed so that there is a continuum in distribution between normal subjects, psychotic prone subjects, and schizophrenic patients. As such, they may be relatively sensitive to degrees of psychopathology in important areas of functioning. These may, in some way, be associated with areas of deficit in neuropsychology or neuroanatomy. Moreover, they may be used to subtype patients a more fine grained matter than the typical positive-negative way.

Project Description:

Objectives: It is hypothesized that some patients will show high scores on magical ideation and perceptual aberration, other patients will show high scores on the anhedonia scales only, and some patients will show high scores on both. These clusters of patients will be examined in terms of their neuropsychological performance, neuroanatomy, and brain metabolism.

Methods Employed: All patients entering the program will be administered the Chapman Scales. They are a self-report inventory. All statements are of the "True/False" variety. It takes about 20-30 minutes to administer.

Major Past Findings: The study is in progress.

Significance to Mental Health Research: This study may provide a means to better understand the relationship between symptoms, neuropsychology, and neuropathology or neuropathophysiology. This relationship has been surprisingly obscure.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02436-01 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Combined Sinemet and Neuroleptic Treatment in Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David G. Daniel, M.D., Senior Staff Fellow, CBDB, NIMH

Joel E. Kleinman, M.D., Ph.D., Deputy Chief, CBDB, NIMH; Daniel R. Weinberger, M.D., Chief, CBDB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies; Section on Neuropathology Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths

TOTAL MAN-YEARS:

.33

PROFESSIONAL:

.33

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This protocol has been approved by the NIMH ICRS. In order to study the effects of augmenting central dopamine activity on brain function and "negative" or "deficit" symptoms in patients with schizophrenia, we have designed a double-blind placebo-controlled crossover trial of combined I-Dopa and molindone or haloperidol, lasting 16 weeks. Throughout the study patients will receive daily BPRS ratings, and twice weekly NSRS and AIMS ratings. At equivalent times during the active and placebo periods patients will undergo measurement of serum prolactin and growth hormone levels, lumbar puncture, computerized brain electrical activity measurement, and cerebral blood flow measurement either by SPECT or rCBF.

Project Description:

Objectives: The purpose of this study was to elucidate the effects of augmenting central dopaminergic activity on brain function and negative symptoms in patients with schizophrenia. The further objectives were to determine the effects on positive and negative symptoms of treating patients simultaneously with an antipsychotic (i.e., molindone) that selectively blocks subcortical D2 receptors (which are thought to be important in producing positive symptoms) and a second medication that stimulates D1 receptors.

Methods Employed: We designed a double-blind placebo controlled cross-over trial of combined L-DOPA and malindone lasting for 16 weeks. Throughout the study, patients will receive daily BPRS ratings and twice weekly NSRS and AIMS ratings.

Major Past Findings: This work is to begin in the fall of 1988.

New Findings: Work has not begun yet.

Significance to Mental Health Research: Negative symptoms are frequently resistant to treatment with neuroleptics. Elucidation of the effects of stimulating D1 receptors is preferentially to D2 receptors may help in the development of future treatments with fewer side effects than.

Proposed Course of Project: Up to 20 patients will be studied. Throughout the study, patients will receive daily BPRS ratings and twice weekly NSRS and AIMS ratings. At equivalent times during the afternoon placebo periods, patients will undergo measurement of serum prolactin and growth hormone levels, lumbar puncture, computerized brain electrical activity measurement and cerebral blood flow measurement either by SPECT or rCBF.

Publications:

Study has not begun.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02437-01 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hydergine In the Treatment of the Negative Symptoms of Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David G. Daniel, M.D., Senior Staff Fellow, CBDB, NIMH

Craig N. Karson, M.D., Staff Psychiatrist, CBDB, NIMH; Darrell G. Kirch, M.D., Medical Director, Neuropsychiatric Research Hospital, NIMH; Daniel R. Weinberger, M.D., Chief, CBDB, NIMH

COOPERATING UNITS (If any)

Neuropsychiatric Research Hospital

LAB/BRANCH

Clinical Brain Disorders Branch; Neuropsychiatric Research Hospital

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths

TOTAL MAN-YEARS:

.33

PROFESSIONAL:

.33

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hydergine (DHE), a combination of dihydrogenated ergot alkaloids is the most widely used "cognitive enhancing" drug prescribed for amelioration of the symptoms of senile dementia. A number of reports have described cognitive deficits, particularly in prefrontal cortex functions in patients with chronic schizophrenia. Ten patients who fulfilled DSM-111 criteria for chronic schizophrenia were stabilized for at least six weeks on either active or placebo neuroleptics. Subsequently, each patient received, in addition to their neuroleptics, two weeks of placebo DHE, followed by 4 weeks of 6 to 9 mg per day of active DHE, followed by 4 weeks of placebo DHE. All patients and raters were blind to the active or placebo status of medications. Patients were evaluated daily on the brief psychiatric rating Scale (BPRS) with the last 2 weeks of the peak active dose period and last 2 weeks of each placebo period used for statistical analysis. Neuropsychological testing was performed during the last 2 weeks of the peak active dose period and during the final 2 weeks of one of the placebo periods. Preliminary analysis suggests that Hydergine did not have a statistically significant effect on either neuropsychological testing or the BPRS.

Project Description:

Objectives: The mechanism of cognitive deficits in schizophrenia is still poorly understood. Hydergine, which is a partial agonist for norepinephrine and dopamine, has been found to produce modest improvement in intellectual deterioration in patients with Alzheimer's disease. We set out to determine if hydergine would have a similar affect on intellectual deterioration seen in schizophrenia.

Methods Employed: Ten patients who fulfilled DSM-III-R criteria for chronic schizophrenia, were stabilized for at least six weeks on either active or placebo neuroleptics. Subsequently, each patient received, in addition to their neuroleptics, four weeks of active and placebo DHE. Patients were evaluated daily on the brief psychiatric rating scale and were evaluated during both the placebo and active periods with neuropsychological testing.

Major Past Findings: This work began in the spring of 1986. We did not report any findings before the current period.

New Findings: Preliminary analysis suggested hydergine did not have a statistically significant effect on either neuropsychological testing or the BPRS.

Significance to Mental Health Research Findings: Elucidation of the psychopharmacological mechanism of cognitive deficits and behavioral abnormalities in schizophrenia may lead to development of more effective and safer drugs for the treatment of schizophrenia.

Proposed Course of the Project: This project has now been completed and is in the data analysis stage.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02438-01 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Unimodal vs Bimodal Distribution of Ventricular Size in Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David G. Daniel, M.D., Senior Staff Fellow, CBDB, NIMH

Terry Goldberg, Ph.D., Special Expert, CBDB, NIMH; Daniel R. Weinberger, M.D., Chief, CBDB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths

TOTAL MAN-YEARS:

.33

PROFESSIONAL:

.33

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Significant clinical and laboratory differences have been reported between schizophrenic patients with large and small-ventricular size. If large ventricles are a marker for a distinct subtype of schizophrenia, then the large ventricles might be expected to form a distinct cluster apart from the main body of values. In order to assess this question of unimodality versus bimodality of ventricular size in schizophrenia we reviewed all published English language VBR studies in which individual data points were available (schizophrenics: $n = 691$, medical controls: $n = 205$, unselected volunteers: $n = 160$). We plotted the frequency distribution of raw VBRs, Z-transformed VBRs, and VBRs transformed utilizing the group mean for the volunteers to correct for small inter-study differences in measurement technique. In each case distributions were unimodal and skewed to the right for schizophrenic patients. These results do not support the notion of bimodality of ventricular size in schizophrenia. The mean raw VBR for schizophrenic patients (mean = 6.75) was significantly larger ($F = 82.5$, $p < .0001$) than that of medical controls (mean = 3.90) and unselected volunteers (mean = 3.79).

Project Description:

Objectives: The purpose of this project was to determine if enlarged ventricula size is a marker for a distinct subtype of schizophrenia.

Methods Employed: If large ventricles are a marker for a distinct subtype of schizophrenia, then the large ventricles might be expected to form a distinct cluster apart from the main body of values. In order to assess this question, we reviewed all published English language VBR studies in which individual studies in which individual data points were available (schizophrenic N = 691, medical controls n = 205, and selected volunteers n = 160). The frequency distribution of raw VBRs, Z transformed VBRs, and VBRs transformed utilizing the group mean to the volunteers to correct for small interstudy differences in measurement technique were plotted.

Major Past Findings: This work began in the fall of 1987. We did not report any findings before the current period.

New Findings: In each case, distributions were unimodal and skewed to the right for schizophrenic patients. These results did not support the notion of bimodality in ventricular size in schizophrenia. The mean raw VBR for schizophrenic patients (mean = 6.75) was significantly larger ($F = 82.5$, $p > .0001$) than that of medical controls (mean = 3.90) and unselected volunteers (mean = 3.79).

Significance to Mental Health Research: These findings suggest that enlarged ventricles are not a marker for biologically distinct subtypes of the schizophrenia. The findings suggest that the distribution of ventricular size exists in a simple continuum.

Proposed Course of Project: This project has been completed.

Publications:

Results of this study are currently being compiled for submission for publication.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02439-01 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Apomorphine and Cerebral Blood Flow in Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David G. Daniel, M.D., Senior Staff Fellow, CBDB, NIMH

Karen F. Berman, M.D., Staff Psychiatrist, CBDB, NIMH; Ralph Fawcett, M.D., Medical Staff Fellow, NPB, NIMH; Daniel R. Weinberger, M.D., Chief, CBDB, NIMH

COOPERATING UNITS (if any)

Neuropsychiatry Branch

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths

TOTAL MAN-YEARS:

.33

PROFESSIONAL:

.33

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We conducted a double-blind placebo controlled study of the effects of .005 mg/kg of apomorphine, a direct acting dopamine agonist, on regional cerebral blood flow (Xe-133 rCBF) during a prefrontal cortex activation procedure in six drug-free schizophrenic patients. Following a simple Numbers Matching control task, each subject received either placebo or active apomorphine (SQ) and then performed a prefrontal activation task (Wisconsin Card Sort). In each patient with schizophrenia, apomorphine increased relative prefrontal flow (paired $t = 2.93$, $n = 6$, $p = .03$) during the prefrontal cortex activation procedure. In addition, during the prefrontal activation task patients showed greater increases in relative prefrontal flow compared to the control task with apomorphine than with placebo (paired $t = 3.31$, $n = 6$, $p = .02$). The results suggest that in schizophrenia enhanced prefrontal dopamine increases relative rCBF in the prefrontal cortex, an area implicated by recent cognitive and physiological studies in the pathophysiology of schizophrenia.

Project Description:

Objectives: Recently, we found a direct correlation in schizophrenia between CSF HVA, the major dopamine metabolite in the CSF, and activity of the prefrontal cortex during a prefrontal activation task. We undertook the current study to further elucidate the role of dopaminergic neurotransmission in the activity of the prefrontal cortex.

Methods Employed: In a double-blind placebo controlled crossover design .005 mg/kg SQ of apomorphine was administered to six drug-free schizophrenics before a prefrontal activation procedure.

Major Past Findings: The previous study found a direct relation to cerebral spinal fluid HVA, during a prefrontal specific task in patients with schizophrenia.

New Findings: In each patient with schizophrenia, apomorphine increased relative prefrontal flow ($p = .03$) during the prefrontal activation tasks.

Significance to Mental Health Research: The results suggest that dopamine increases relative rCBF in the prefrontal cortex, and that deficits in prefrontal activation and schizophrenia may be reversible with pharmacotherapy.

Proposed Course of Project: This project has been completed.

Publications:

Results are currently being compiled publication.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02440-01 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Relationship of Occipital Skull Asymmetry to Brain Parenchymal Measures in Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David G. Daniel, M.D., Senior Staff Fellow, CBDB, NIMH

Michael S. Myslobodsky, M.D., Visiting Scientist, CBDB, NIMH; Richard Coppola, D.Sc., Senior Engineer, CBDB, NIMH; Daniel R. Weinberger, M.D., Chief, CBDB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths

TOTAL MAN-YEARS:

.40

PROFESSIONAL:

.40

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

It has long been hypothesized but never proven that an organic brain injury early in life could predispose to schizophrenia.

One approach to this question is to search for a correlate of brain pathology that itself could be relegated in time to early development. Skull growth is approximately 75% completed by 2 years of age with the final period of rapid growth occurring at puberty. Since brain and cranial development are closely linked, if pathology to the brain occurred early enough in life, it could conceivably affect skull architecture. The occipital bone depth (OBD) and the occipito-median angle (OMA) were assessed bilaterally in the CT scans of 50 chronic schizophrenics (DSM-III-R) and the asymmetry index (AI) ($(R-L/R + L \times 100)$) was computed. The chief findings of the study are that in patients with schizophrenia: 1) occipital skull asymmetry appears to be a marker which covaries with parenchymal brain asymmetries; 2) the mean OBD asymmetry index for the 50 patients with schizophrenia in our study was not significantly different from that of 35 normal control subjects described in an earlier study; 3) The right temporal lobe was wider in a large majority of our subjects; 4) The left occipitopetalia was more prominent and the left parieto-occipital lobe wider in most of the patients. Both a relatively wider right parieto-occipital area and a more protruberant right occipito-petalia were associated with crossed dominance in this sample; and 5) Positive OBD asymmetry indices (flatter left occipital bone) correlate with increased prefrontal cortical markings.

Project Description:

Objectives: It has been hypothesized that an organic brain injury early in life could predispose to schizophrenia. We attempted to approach this question by searching for a correlative brain pathology that itself could be relegated in time to early development. Since skull growth is approximately 75% completed by two years of age, and since brain and cranial development are closely linked they hypothesized that if pathology to the brain occurred early enough, it conceivably effect skull architecture.

Methods Employed: The occipital bone depth and the occipital median angle were assessed bilaterally in the CT scans of 50 chronic schizophrenics. An asymmetry index $CR-L/R + L \times 100$ was computed.

New Findings: In patients with schizophrenia: 1) occipital skull asymmetry appeared to be a mark -- which covaried with parenchymal brain asymmetry 2) the main occipital brain depth asymmetry index for 50 patients with schizophrenia in our study was not significantly different from that of 35 normal control subjects described in earlier studies, 3) right temporal lobe was wider in a large majority of our subjects; 4) the left occipitopetalia was more prominent in the left parietal occipital lobe; wider in most of the patients. Both are relatively wider right parieto-occipito area and a more protruberant right occipitopetalia were associated with crossed dominance in this sample and five positive occipital bone depth asymmetry and disease (flatter occipital bone) correlated with increased prefrontal cortical markings.

Significance to Mental Health Research: Delineation of the pathological changes in schizophrenia as well as their time course of development will hopefully permit further investigation of their etiology and possible therapeutic interventions.

Proposed Course of Project: This project has been completed.

Publications:

The manuscript is being prepared for submission.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02441-01-CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Density in Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David G. Daniel, M.D., Senior Staff Fellow, CBDB, NIMH

Debra Kostlanovsky, M.D. (Guest worker); E. Kim, M.D. (Guest worker); Terry Goldberg, Ph.D., Special Expert, CBDB, NIMH; Manuel Casanova, M.D., CBDB, NIMH; Joel E. Kleinman, M.D., Ph.D., Deputy Chief, CBDB, NIMH; Daniel R. Weinberger, M.D., Chief, CBDB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

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INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths

TOTAL MAN-YEARS:

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PROFESSIONAL:

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OTHER:

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CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Several previous studies have demonstrated differences in regional brain density (as derived from CT scan attenuation values) between patients with schizophrenia and normal controls. Interpretation of these studies has been hindered by methodological shortcomings such as failure to control for head size, scanner calibration differences and other confounding variables. The present study offered methodological advances over earlier studies by controlling for head size and normalizing the attenuation values for each scan to an internal standard. CT attenuation values in multiple brain regions in 20 patients with chronic schizophrenia were compared with those of 20 age and sex matched controls. No significant differences emerged between the schizophrenics and normal controls. The results confirm the importance of controlling for artifacts in analysis of CT scan attenuation values and raise questions about the validity of regional CT attenuation values in detecting subtle anatomical abnormalities in schizophrenia.

Project Description:

Objectives: Previous studies have demonstrated differences in regional brain density between patients with schizophrenia and normal controls. However, many of these studies have been hindered by methodological shortcomings such as further control for head size, scanner, calibration differences and other confounding variables. The purpose of the present study is to determine if the findings of previous studies could be replicated when the method was adjusted to control for head size and scanner calibration differences.

Methods Employed: CT attenuation values in multiple brain regions and 20 patients with chronic schizophrenia were compared with those of 20 age and sex matched controls. The present study controlled for head size and normalized the attenuation values for each scan to an internal standard.

Major Past Findings: Work on this study began in January of 1988. We did not report any findings before the current period.

New Findings: No significant differences emerged between the schizophrenics and normal controls. The results confirmed the importance of controlling for artifacts and analysis of CT scan attenuation values and raised questions about the validity of regional CT attenuation values in detecting subtle anatomical abnormalities in schizophrenia.

Proposed Course of Project: This project has been completed.

Publications:

Results are currently being written for publication.

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